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Research Article

A Novel Validated HS-GC-MS method for the Trace Level Determination of Acetyl Chloride as Isopropyl Acetate in Various Anti-cancer Drug Substances

Srivalli Kasina[1*], Annapurna Nowduri[2], Ramdas Chavakula[1]

[1]*Aurobindo Pharma Limited Research Centre-II, Kandi Mandal, Sangareddy, Telangana, India.*
[2]*Andhra University College of Engineering, Visakhapatnam, Andhra Pradesh, India.*

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ABSTRACT

A novel, simple and precise headspace gas chromatography-mass spectrometry (HS-GC-MS) method was developed and validated for the determination of acetyl chloride (AC) in various anti-cancer drug substances like bendamustine HCl, Ibrutinib, trifluridine and abemaciclib drug substances. In this methodology, AC reacts with isopropyl alcohol and is converted into isopropyl acetate. Hence AC is quantified as Isopropyl acetate. The lower level of detection was achieved on the capillary GC column (DB-624, Fused silica capillary column; 30 m length; 0.32 mm internal diameter, coated with 6% Cyanopropylphenyl and 94% dimethyl polysiloxane stationary phase of 1.8 µm film thickness) with electron impact ionization (EI) technique by GC-MS in selective ion monitoring (SIM) mode. The mass ions were selected for the quantifier is 63 and the qualifier is 87 for isopropyl acetate. The performance of the method was assessed by evaluating the specificity, linearity, sensitivity, precision, accuracy and robustness experiments. The established limit of detection and limit of quantification values for the AC were 0.1 and 0.3 µg/g, respectively. This developed method was found to be linear with a correlation coefficient is greater than 0.999 for all four drug substances. The average recoveries of AC in bendamustine HCl, ibrutinib, trifluridine and abemaciclib were obtained 106.1, 109.0, 97.4 and 101.6%, respectively. The proposed method was validated successfully as per ICH guidelines. Hence, the proposed method can be routinely used for the quantification of AC in various anti-cancer drug substances.

Introduction

Bendamustine hydrochloride (BMH) is described chemically known as 4-[5-[bis-(2-chloroethyl)amino]-1-methylbenzimidazol-2-yl]butanoic acid hydrochloride is an active nitrogen mustard.[1-2] The chemical formula of Bendamustine HCl is $C_{16}H_{21}Cl_2N_3O_2$. HCl and its molecular Weight is 394.72 g/mol. BMH is used for treating various hematological malignancies. In March 2008, it was approved by the US Food and Drug Administration (FDA) for the treatment of chronic lymphocytic leukemia and sarcoma,[3,4] multiple myeloma and rituximab-refractory indolent B-cell non-Hodgkin lymphoma (NHL).[5] It combines the pharmacological actions of alkylating agents and antimetabolites.[6] BMH is a bifunctional mechlorethamine derivative containing a purine-like benzimidazole ring. Mechlorethamine and its derivatives form electrophilic alkyl groups. These groups form covalent bonds with electron-rich nucleophilic moieties, resulting in interstrand DNA crosslinks. The bifunctional covalent linkage can lead to cell death activation of DNA damage stress response and apoptosis, inhibition of mitotic checkpoints, and induction of mitotic catastrophe. BMH was developed by Cephalon in the United States as *Treanda*.[7,8] It exhibits cytotoxic activity against human ovarian and breast cancers *in-vitro*.[9,10] BMH is a white, watersoluble microcrystalline powder with amphoteric properties. It acts as an alkylating agent causing intra-strand and inter-strand cross-links between DNA bases.

***Corresponding Author:** Dr. Srivalli Kasina

Address: Aurobindo Pharma Limited Research Centre-II, Indrakaran village, Kandi Mandal, Sangareddy, Telangana, India

Email ✉**:** sivakrishna.sri2008@gmail.com

Tel.: +91-8008923369

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Ibrutinib (IBR) is chemically known as 1-[(3R)-3-[4-amino-3-(4-phenoxyphenyl)pyrazolo[3,4-d]pyrimidin-1-yl]piperidin-1-yl]prop-2-en-1-one. Ibrutinib's molecular weight and formula are 440.50 g/mol and $C_{25}H_{24}N_6O_2$, respectively. IBR is an anti-cancer drug that acts as an irreversible, potent inhibitor of Burton's tyrosine kinase.[11,12] IBR belongs to a class of tyrosine kinase inhibitors for the remedy of B-cell malignancies. IBR was chosen as a targeted covalent drug and presents a promising activity in B cell malignancies.[13,14] Ibrutinib was approved by the FDA on November 13, 2013 for the treatment of mantle cell lymphoma (MCL).[15,16] On February 12, 2014 FDA extended the approval for the treatment of B-cell cancers like Waldenstrom's macroglobulinemia (WH), mantle cell lymphoma[17-18] and chronic lymphocytic leukemia[19] because of Burton's tyrosin kinase (BTK) protein, which is important in B cells is covalently bonded the IBR drug. Basically, IBR works by stopping or slowing the escalation of cancer cells. It is available as Imbruvica capsules for oral administration containing 140 mg Ibrutinib as the active ingredient.

Trifluridine (TFD) is a nucleoside analog antiviral fluorinated thymidine analog with potential antineoplastic activity. TFD is incorporated into DNA synthesis, inhibition of protein synthesis and apoptosis. TFD is an antineoplastic nucleoside analog discovered by Heidelberger and others at the University of Wisconsin as a drug that inhibits thymidylate synthetase similarly to existing fluoro pyrimidines but exerts a growth inhibitory effect mainly by being incorporated into DNA of tumor cells. Lonsurf is a novel oral nucleoside antitumor agent that consists of trifluridine and tipiracil drug substances. Lonsurf is specifically indicated for patients with metastatic colorectal cancer. Trifluridine is chemically 1-[(2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-5-(trifluoromethyl)pyrimidine-2,4-dione.[20-26] TFD is metabolized by the enzyme thymidine phosphorylase to 5-trifluoromethyl-2,4(1H,3H)-pyrimidinedione (FTY), and also by glucuronidationis. Trifluridine's molecular weight and formula are 296.2 g/mol and $C_{10}H_{11}F_3N_2O_5$.

Abemaciclib (ABM) is an orally administered small molecule inhibitor of cyclin-dependent kinases (CDKs) 4 and 6.[27] It has reportedly been effective as a first-line treatment for hormone receptor-positive (HR-positive), HER2-negative advanced breast cancer when combined with a fulvestrant or nonsteroidal aromatase inhibitor.[28–30] On September 28, 2017, FDA granted approval of ABM treatment under the market name Verzenio for the treatment of HR-positive and HER2-negative advanced or metastatic breast cancer that has progressed after unsuccessful endocrine therapy. On the other hand, hematological toxicity and diarrhea, common adverse drug reactions (ADRs) of ABM, are known to results in drug intolerance in patients.[31,32] Recently, some ADRs of ABM have been reported to be dose-dependent.[33] However, the pharmacokinetics of ABM vary largely among individual patient[34] and have been reported to be affected by concomitant use of drugs such as CYP3A modulators.[35] Following oral treatment in HR-positive, HER2-negative breast cancer patients, ABM demonstrated increased progression-free survival rates and objective response rates. ABM has been used in trials studying the treatment of melanoma, lymphoma, neoplasm, solid tumor, and glioblastoma. Abemaciclib is described chemically known as *N*-[5-[(4-ethylpiperazin-1-yl)methyl]pyridin-2-yl]-5-fluoro-4-(7-fluoro-2-methyl-3-propan-2-ylbenzimidazol-5-yl)pyrimidin-2-amine. The molecular weight and formula of abemaciclib are 506.59 g/mol and $C_{27}H_{32}F_2N_8$.

Acetyl chloride (AC) is a reactive compound that is often used as a two-carbon building block reagent given its multifunctional properties. The wide use of AC in the pharmaceutical industry as an acylating agent in the synthesis of active pharmaceutical ingredients (API) has shown to be a critical reagent.[36–38]

Glutaric anhydride is one of the key starting materials in BMH drug substance synthesis. Acetyl chloride is used as a reagent in the synthesis of glutaric anhydride. Acryl chloride is one of the key starting material in IBR drug substance synthesis. Acetyl chloride is used as a reagent in the synthesis of acryl chloride. Acetyl chloride is used as a reagent in the preparation of intermediates stages of both TFD and ABM drug substance synthesis. Although limited information is available regarding AC's genotoxicity and carcinogenicity, it can be categorized as an alerting structure for genotoxic potential. The potential genotoxicity of AC allows for a class 3 impurity based on Muller's classification.[39] The class 3 assignment for AC is due to the acyl halide acting as a strong electrophilic agent known to react with DNA. The high reactivity of AC allows a reaction with residual moisture and solvents present in either the initial reaction mixture or subsequent steps to form potential degradation species.

Acetyl chloride is structurally alert highly reactive potential genotoxic impurity (PGI) reported as a mutagenic impurity in the ICH M7 guideline.[40] The presence of residual AC in these four drug substances i.e. BMH, IBR, TFD and ABM must be checked and controlled as per European Medicines Agency (EMA), International Council for Harmonization and Food and Drug Administration (FDA) guidelines.[41,42] EMA and FDA guidelines proposed using the "threshold of toxicological concern" (TTC) concept to limit genotoxic/carcinogenic impurities. A TTC-based acceptable in-take of a mutagenic impurity of 1.5 µg per person per day is considered to be associated with a negligible risk (theoretical excess cancer risk of <1 in 100,000 over a lifetime of exposure) and can in general be used for most pharmaceuticals as a default to derive an acceptable limit for control. This approach would usually be used for mutagenic impurities present in pharmaceuticals for long-term treatment (>10 years)

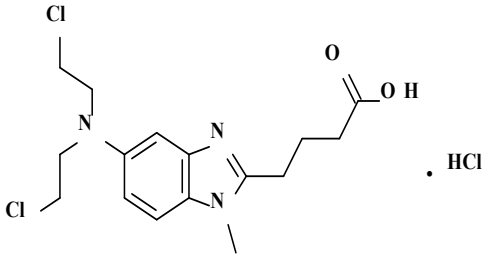


Fig. 1: (a) Chemical structure of bendamustine HCl (BMH)

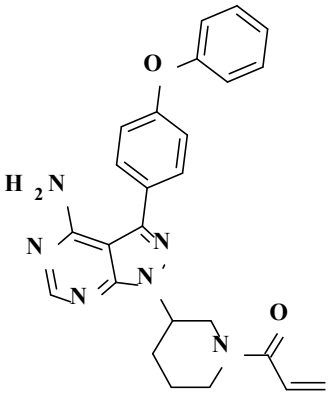


Fig. 1: (b) Chemical structure of ibrutinib (IBR)

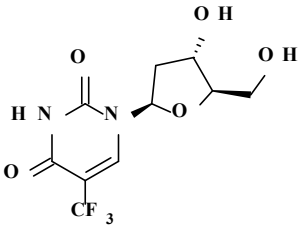


Fig. 1: (c) Chemical structure of trifluridine (TFD)

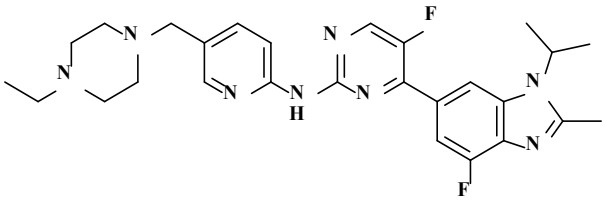


Fig. 1: (d) Chemical structure of abemaciclib (ABM)

Fig. 1: (e) Chemical structure acetyl chloride (AC)

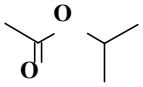


Fig. 1: (f) Chemical structure isopropyl acetate

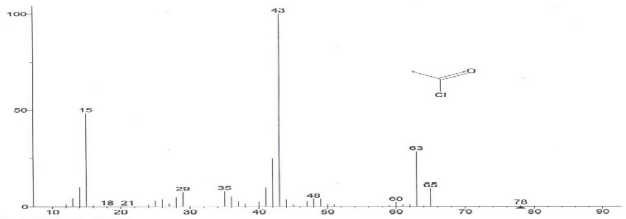


Fig. 2(a): Mass spectrum of acetyl chloride

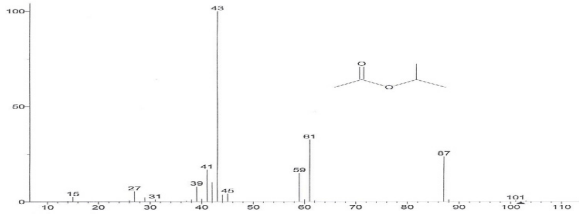


Fig. 2(b): Mass spectrum of isopropyl acetate

and where no carcinogenicity data are available (Classes 2 and 3).[40] The concentration limits in ppm of genotoxic impurity in drug substances derived from the TTC can be calculated using the equation based on the expected daily dose to the patient.

Concentration limit (ppm)	TTC [µg/day]	
	dose [g/day]	

The maximum daily dose of BMH is 150 mg/m^2 body surface. Assuming a standard body surface of 1.874 m^2 for a height of 1.70 m and weight of 76 kg the maximum daily dose will be 281 mg (For calculation of body surface area (BSA) the DuBois formula was used). So the limit of AC for BMH is 5.4ppm (1.5/280*1000) justified. The maximum daily dose of IBR is 560 mg orally once daily. So the limit of AC for IBR is 2.7 ppm (1.5/560*1000). The maximum daily dose of TFD is 80 mg taken orally twice daily. So the limit of AC for TFD is 9.4 ppm (1.5/160*1000). The maximum daily dose of ABM is 200 mg taken orally twice daily. So the limit of AC for ABM is 3.8 ppm (1.5/400*1000).

To the best of our knowledge, no GC, GC-MS or any other analytical technique methods are available in the literature for the trace level quantitative determination of acetyl chloride impurity in various drug substances. This research paper describes a fast, reliable and validated HS-GC-MS method that is capable of determining AC in various drug substances. In this method, acetyl

chloride has been derivatized with Isopropyl alcohol and determined as the derivative of acetyl chloride, i.e., Isopropyl acetate. Further, the method is validated to comply with the requirements of ICH Validation guidelines.[43] The chemical structures of BMH, IBR, TFD and ABM and AC and derivative of AC i.e. Isopropyl acetate are shown in Figs 1(a) to (f). The GC-MS spectrums of AC and derivatized peak i.e. Isopropyl acetate were shown in Figs. 2(a) and (b).

Materials and Methods

Chemicals and Reagents

Acetyl chloride obtained from AVRA Synthesis Private Ltd. with 99.56% purity, Isopropyl alcohol and 1-Methyl-2-pyrrolidinone (Analytical grade) procured from Rankem, India. The four investigated drug substances bendamustine HCl, Ibrutinib, trifluridine and Abemaciclib were gifted from APL Research Centre laboratories (A division of Aurobindo Pharma Ltd., Hyderabad). Acetaldehyde, methanol, ethanol, isopropyl alcohol, chloroform, benzene, toluene, acetonitrile, acetone, dichloromethane, methyl tert-butyl ether, etc. solvents were procured from Sigma Aldrich (Steinheim, Germany) which were used in various drug substances.

Table 1: Gas chromatograph conditions for Acetyl chloride analysis

Instrument	*Agilent 7890B*		
Column	DB-624, 30m × 0.32mm I.D. × 1.8µm Film thickness		
Carrier gas	Helium		
Injector temperature (°C)	240°C		
Injection type	Headspace		
Column oven program	Heating rate (°C/min)	Initial temperature (°C)	Hold time (min)
		40	4
	20	220	7
Flow rate (mL/min)	2.0		
Split ratio	1 : 10		
Run time (min)	20min		
Headspace conditions			
Oven temperature	:	80°C	
Loop temperature	:	90°C	
Transfer line temperature	:	110°C	
Vial equilibration time	:	20 minutes	
Pressure equilibration time	:	0.2 minutes	
Loop equilibrium time	:	0.05 minutes	
Injection duration	:	1.0 minute	
Injection volume	:	1.0 mL (loop) Auto injection system	
Shake vials while in oven	:	71 shakes/min (level-5)	
Fill Pressure	:	14.2 psi	
GC cycle	:	30 minutes.	
Vial size	:	20 mL	

Table 2: Gas chromatography mass spectrometer conditions for Acetyl chloride analysis.

Instrument	*Agilent GCMS-5977A and GCMS-5977B Single Quad MS*
MSD transfer line temperature	250°C
MS Source temperature	230°C
MS Quad temperature	150°C
Function type	SIM (selective ion monitoring)
Gain factor	3

Table 3: SIM Time segments

Solvent delay time	*Group name*	*Resolution*	*Mass(m/z)*	*Dwell time(ms)*
3.0	Isopropyl acetate	Low	61*, 87**	100, 100

*Quantifier ion
** Qualifier ion

Timed MS Detector:
The MS must be Detector Off after 8.0 min.

Instrumentation

The analysis was carried out on the Agilent GCMS-5977A and GCMS-5977B gas chromatography equipped with 7890B GC System auto sampler and data handling system having Mass Hunter solution software (Make: Agilent Technologies, Santa Clora, CA, USA). The instrument was run in EI mode. DB-624, (30m × 0.32 mm I.D, 1.8 µm film thickness, Agilent Technologies, USA) column consists of 6% cyanopropylphenyl and 94% dimethyl polysiloxane as a stationary phase was used for the study. Chromatographic method conditions used were as follows [Tables 1-3].

Preparation of standard solutions

Diluent

Isopropyl alcohol:1-Methyl-2-pyrrolidinone in the ratio of 1:9 v/v.

Standard stock solution

Accurately weigh 67.5 mg of acetyl chloride standard into a 50 mL of volumetric flask half-filled with diluent and make up to the volume with diluent and mix well. Transfer 0.5 mL of this solution into a 25 mL volumetric flask and make up the volume with diluent. (Acetyl chloride weights are varying w.r.t limits respective to various drug substances)

Standard solution

Transfer 1.0 mL of above the standard stock solution into a 50 mL volumetric flask and dilute to volume with diluent and mix well. (This standard solution for Bendamustine Hydrochloride).

Introduce 1-mL of standard solution into a headspace vial and close the vial with butyl rubber septa and metallic ring closure.

Blank solution

Introduce 1-mL of diluent into a headspace vial with butyl rubber septa and metallic ring closure.

Sample solution

Accurately weigh about 100 mg of the test sample into a headspace vial, add 1-mL of diluent, and seal the vial with butyl rubber septa and metallic ring closure.

Method Validation

Specificity

As per ICH guidelines,[44] specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The specificity of the developed GC-MS method was verified in the presence of residual solvents like methanol, ethanol, isopropyl alcohol, dichloromethane, chloroform, benzene and toluene which were used in the BMH synthesis process. BMH sample solution (Control sample), BMH drug substance spiked with Acetyl chloride at specification level (5.4 µg/g) (Spiked Sample) and BMH drug substance spiked with acetyl chloride and all other known residual solvents at specification level (All Spiked Sample) were injected into GC-MS to confirm any co-elution of AC as Isopropyl acetate and with any other known residual solvents. Typical GC-MS chromatograms of a control sample, spiked sample and all spiked sample are shown in Fig. 3. Specificity results are shown in Table 4 and these experimental results indicating that acetyl chloride as Isopropyl acetate peak is homogeneous from all other known residual solvents. Similarly, the specificity of the developed HS-GC-MS method was verified in the presence of residual solvents which were used in the IBR synthesis process. IBR sample solution (Control sample), IBR drug substance spiked with AC at specification level (2.7 µg/g) (Spiked Sample) and IBR drug substance spiked with AC and all other known residual solvents at specification level (All Spiked Sample) were injected into GC-MS to confirm any co-elution of AC as Isopropyl acetate and with any other known residual solvents. Typical GC-MS chromatograms

Table 4: Specificity experiments results

S.No	*Name of the drug substance*	*Sample ID*	*IP acetate Response (Area counts)*	*AC content as IP acetate (µg/g)*	*Recovery (%)*
		Control sample	1964	0.32	---
1	Bendamustine HCl	Spiked sample	38264	6.19	106.0
		All spiked sample	37983	6.19	105.2
		Control sample	Not detected	Not detected	Not detected
2	Ibrutinib	Spiked sample	16691	2.95	109.3
		All spiked sample	16926	3.00	110.7
		Control sample	1278	0.19	0.19
3	Trifluridine	Spiked sample	61751	9.27	98.2
		All spiked sample	61842	9.33	98.3
		Control sample	359	Not detected	Not detected
4	Abemaciclib	Spiked sample	20421	3.79	99.2
		All spiked sample	20246	3.76	98.4

Table 5: LoD/LoQ and Linearity experiments results

Statistical parameters	BMH	IBR	TFD	AMB
	Results			
Correlation coefficient	0.9999	0.9996	0.9995	0.9995
Concentration range (µg/g)	0.31 – 8.42	0.30 – 4.08	0.31 – 13.97	0.30 – 5.64
Calibration points	7	7	7	7
Intercept	419.8718	420.5764	1467.4599	337.3041
Slope(S)	5931.5340	5914.0237	5914.5048	5552.6383
Limit of detection (µg/g)	0.1	0.1	0.1	0.1
Limit of quantification (µg/g)	0.3	0.3	0.3	0.3
Precision for Limit of Detection (%RSD)	3.1	1.3	4.2	3.8
Precision for Limit of Quantification (%RSD)	0.7	1.0	1.2	0.8

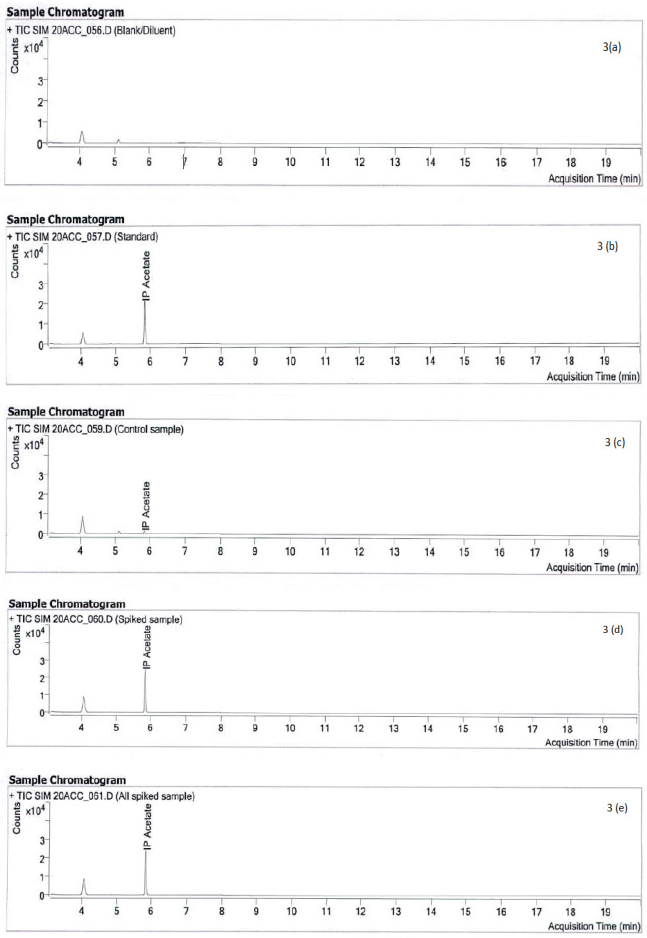


Fig. 3: Typical GC-MS chromatograms of the blank, standard, control sample, spiked sample and all spiked sample for BMH

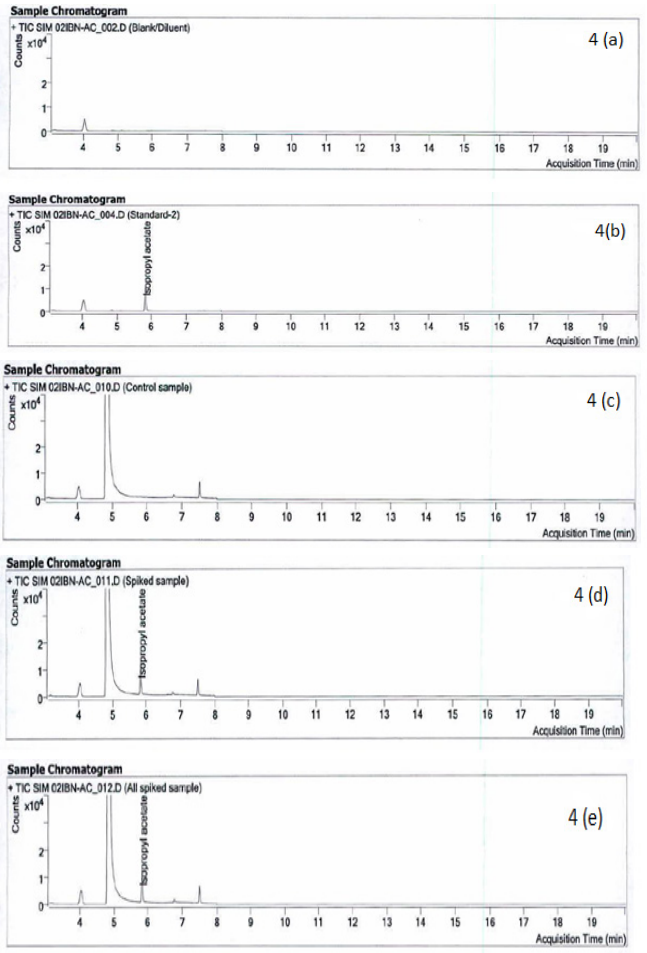


Fig. 4: Typical GC-MS chromatograms of the blank, standard, control sample, spiked sample and all spiked sample for IBR

of a control sample, spiked sample and all spiked sample are shown in Fig.4. Specificity results are shown in Table 4 and these experimental results indicating that AC as isopropyl acetate peak is homogeneous from all other known residual solvents.

Limit of Detection and Limit of Quantification/ Linearity

The limit of detection (LoD) and quantification (LoQ) values of acetyl chloride were determined as Isopropyl acetate using the S/N ratio evaluation method. The predicted concentrations of LoD and LoQ of AC as Isopropyl acetate were verified for precision by preparing the solutions containing at about predicted concentrations, injecting each six times into GC-MS, and calculating the %RSD of peak areas. The series of solutions were prepared using AC at concentration levels from LoQ to 150% of specification level (5.4 µg/g for BMH, 2.7 µg/g for IBR, 9.4 µg/g for TFD and 3.8 µg/g for AMB) and each solution was analysed and calculating the statistical values like slope, intercept, steyx and correlation coefficient from linearity plot drawn for concentration versus area. The statistical experimental values are shown in Table 5 and typical GC-MS chromatograms of a LoD and LoQ are shown in

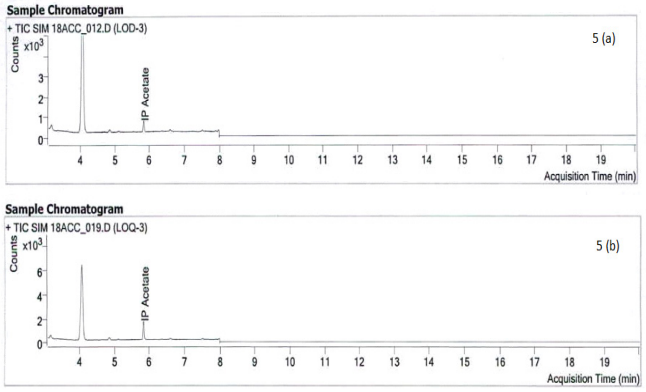
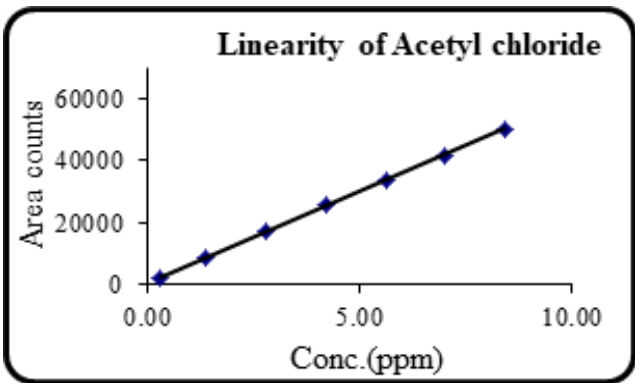


Fig. 5: Typical GC-MS chromatograms of the LoD and LoQ

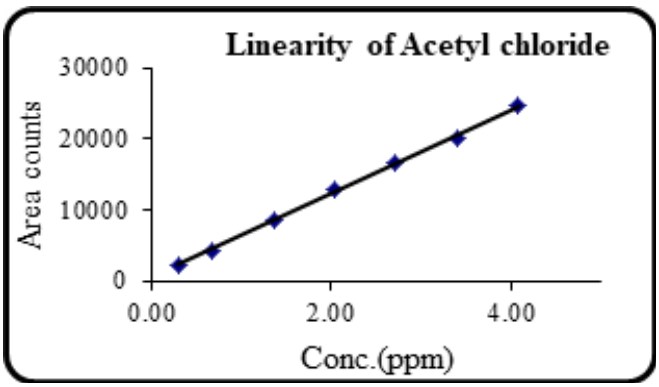
Figs. 5(a) and (b). The linearity plot of various drug substances has been presented in Figs. 6(a) to (d).

Precision

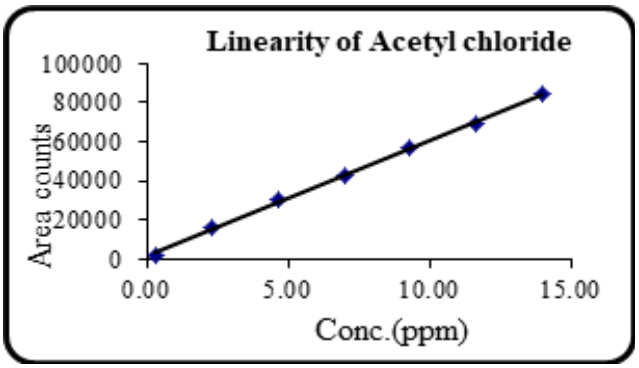
The system precision of the method was checked by analysing standard solution for six replicates and method precision was checked by preparing the six individual sample solutions by spiking the AC at specification level



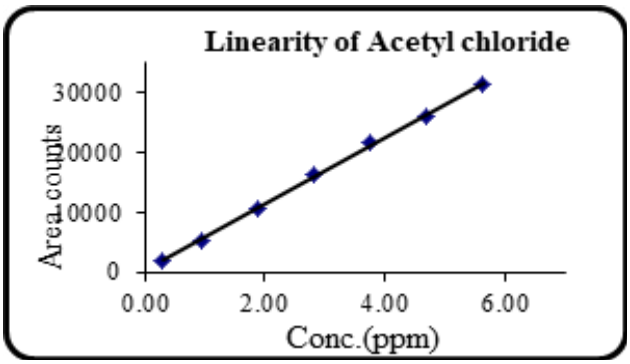
6 (a): Linearity graph of AC in BMH



6 (b): Linearity graph of AC in IBR



6 (c): Linearity graph of AC in TFD



6 (d): Linearity graph of AC in ABM

Fig. 6: Linearity graphs of Acetyl chloride in various drug substances

Table 6(a): Precision experiments results for BMH

Injection ID	*System Precision*	*AC as Isopropyl acetate content, µg/g*	
		Method Precision	*Ruggedness*
1	35470	6.19	6.36
2	35534	6.18	6.57
3	35315	6.19	6.54
4	35348	6.21	6.70
5	35201	6.16	6.36
6	35355	6.20	6.38
Mean	35371	6.19	6.49
SD	118	0.02	0.14
RSD (%)	0.3	0.3	2.2
Overall statistical data (n=12)	Mean	6.34	
SD		0.18	
RSD (%)		2.9	

Table 6(b): Precision experiments results for IBR

Injection ID	*System Precision*	*AC as Isopropyl acetate content, µg/g*	
		Method Precision	*Ruggedness*
1	15481	2.95	2.49
2	15214	2.95	2.43
3	15088	3.00	2.46
4	15308	2.94	2.41
5	15380	2.96	2.40
6	15182	2.92	2.37
Mean	15276	2.95	2.43
SD	143	0.03	0.04
RSD (%)	0.9	1.0	1.6
Overall statistical data (n=12)	Mean	2.69	
SD		0.28	
RSD (%)		10.3	

(5.4 µg/g for BMH, 2.4 µg/g for IBR, 9.4 µg/g for TFD and 3.8 µg/g for ABM) to the drug substance and injected into GC-MS. The results of system and method precision experiments are shown in Table 6.

The precision of the method was studied using repeatability and reproducibility (ruggedness). The system precision was evaluated by analysing six replicates of standard solution for checking the performance of the GC-MS under the chromatographic conditions on the day tested (system precision) and calculated the area of isopropyl acetate from obtained areas for all BMH, IBR, TFD and AMB drug substances. Repeatability and reproducibility of the method was studied

Table 6(c): Precision experiments results for TFD

Injection ID	*System precision*	*AC as Isopropyl acetate content, (µg/g)*	
		Method precision	*Ruggedness*
1	60843	9.27	9.71
2	61946	9.33	9.76
3	60607	9.14	9.81
4	61175	9.18	9.71
5	64882	9.27	9.80
6	60660	9.18	9.68
Mean	61686	9.23	9.75
SD	1641	0.07	0.05
RSD (%)	2.7	0.8	0.5
Overall statistical data (n=12)	Mean	9.49	
SD		0.28	
RSD (%)		2.9	

Table 6(d): Precision experiments results for ABM

Injection ID	*System precision*	*AC as Isopropyl acetate content, (µg/g)*	
		Method precision	*Ruggedness*
1	20648	3.79	3.84
2	20529	3.73	3.84
3	20128	3.76	3.80
4	19984	3.75	3.84
5	20085	3.71	3.77
6	20017	3.77	3.85
Mean	20232	3.75	3.82
SD	283	0.03	0.03
RSD (%)	1.4	0.8	0.8
Overall statistical data (n=12)	Mean	3.79	
SD		0.05	
RSD (%)		1.2	

Table 7: Accuracy experiment results

Name of the drug substance	*BMH*				*IBR*			
Control sample	*0.32 ppm*				*ND*			
	LoQ Level	Level-I (50%)	Level-II (100%)	Level-III (150%)	LoQ Level	Level-I (50%)	Level-II (100%)	Level-III (150%)
*Added (µg/g)	0.31	2.78	5.57	8.39	0.30	1.35	2.71	4.06
*Found (µg/g)	0.36	3.00	5.87	8.82	0.34	1.52	2.97	4.27
Recovery (%)	116.1	107.9	105.4	105.1	113.3	112.6	109.6	105.2
%RSD	2.8	0.6	0.7	0.8	3.0	2.1	0.8	0.8
Name of the Drug substance	*TFD*				*ABM*			
Control sample	*0.19 ppm*				*0.07 ppm*			
	LoQ Level	Level-I (50%)	Level-II (100%)	Level-III (150%)	LoQ Level	Level-I (50%)	Level-II (100%)	Level-III (150%)
*Added (µg/g)	0.31	4.65	9.28	13.91	0.30	1.88	3.76	5.64
*Found (µg/g)	0.28	4.44	9.07	13.77	0.29	2.00	3.70	5.64
Recovery (%)	90.3	95.5	97.7	99.0	96.7	106.4	98.4	100.0
%RSD	4.0	0.8	1.0	0.3	4.0	0.5	0.7	0.2

by analyzing six sample solutions separately. Repeatability was the intra-day variation (Method precision) demonstrated by preparing six sample solutions individually using a single batch of BMH drug substance spiked with AC at about 5.4 µg/g concentration level and content was determined. Similarly demonstrated by preparing six sample solutions individually using a single batch of IBR drug substance spiked with AC at about 2.7 µg/g concentration level, TFD drug substance spiked with AC at about 9.4 µg/g concentration level and ABM drug substance spiked with AC at about 3.8 µg/g concentration level and content was determined.

The intermediate precision was the inter-day variation (Ruggedness) defined as the degree of reproducibility obtained by following the same procedure as mentioned for method precision experiment. Ruggedness of the method was evaluated by preparing six individual sample preparations (same sample used in Method precision experiment) by spiking AC to BMH, IBR, TFD and AMB drug substances injected into different column, instruments and analyst on a different day. The achieved precision experiment results are given in Tables 6(a) to (d)

Accuracy

To prove the recovery for developed GC-MS method, standard addition experiments were conducted in triplicate preparations (BMH, IBR, TFD and ABM drug substance sample solutions were prepared individually and by spiking with AC) at LoQ, 50, 100 and 150% of

specification level and recoveries of AC was determined as Isopropyl acetate. In BMH drug substance, such sample was containing around 0.32 ppm of AC and AC was not detected in IBR drug substance as such sample. In TFD drug substance as such sample was containing around 0.19 ppm of AC and in AMB drug substance as such sample was containing around 0.07 ppm of AC. The obtained recovery values lie between 105.1 and 116.1 for BMH and 105.2 and 113.3 for IBR, 90.3 and 99.0 for TFD and 96.7 and 106.4 for ABM, respectively shows method is accurate. The accuracy experiment results are reported in Table 7.

Robustness

Robustness of the method was evaluated by deliberately altering the method conditions from original method parameters and verifying compliance to the system suitability parameters. The impact of variation of column oven temperature and flow rate of carrier gas on system suitability was conducted. In the robustness verification of test method, one parameter changed while keeping the other unchanged from actual parameter (Tables 8a-c). The study was carried out with respect to Column flow variation of carrier gas initial flow rate ± 10% and column oven initial temperature and ramp temperature ± 2°C and Headspace vial oven temperature ± 5°C as follows. Results of peak areas for Acetyl chloride as Isopropyl acetate is summarized in Table 9 in all BMH, IBR, TFD and ABM drug substances.

Conditions

In each robustness condition, the remaining GC-MS conditions are the same as the test method.

Solution stability

The standard solution and sample solutions were prepared by spiking acetyl chloride at known concentration level for all BMH, IBR, TFD and AMB drug substances and the stability of the solution was tested as freshly prepared and at different intervals with the gap of every one hour and up to 24 hours at ambient conditions. The stability of solution was determined by comparing results with freshly prepared standard and sample solutions. The results indicated that standard and sample solutions were stable for 24 hours at ambent conditions.

Table 8a: Flow variations

Column Flow (ml/min)	
As per Methodology	2.0 mL/min
-10% Flow variation	1.8 mL/min
10% Flow variation	2.2 mL/min

Table 8b: Column oven temperature variations

Column oven temperature			
As per Methodology	40°C (4min)	20° C/min	220°C (7min)
-2°C Column Oven Temperature variation	38°C (4min)	18° C/min	220°C (7min)
+2°C Column Oven Temperature variation	42°C (4min)	22° C/min	220°C (7min)

Table 8c: Headspace vial oven temperature variations

Headspace vial oven temperature	
As per Methodology	80°C
+ 5°C variation	75°C
- 5°C variation	85°C

Table 9: Robustness experiments results

Robustness condition	*Variation*	*Acetyl chloride as Isopropyl acetate Results*			
		BMH	*IBR*	*TFD*	*ABM*
		System suitability criteria (% RSD)			
As per methodology	-	2.4	0.4	2.4	1.1
Flow variation -	-10%	2.7	1.2	0.5	0.5
	+10%	0.8	1.8	2.1	0.4
Temperature variation - Initial Oven and Ramps	-2°C & -2°C/min	0.2	1.2	0.6	0.7
	+2°C & +2°C/min	1.8	1.0	1.6	2.5
Headspace vial oven temperature variation	75°C	3.1	1.3	1.2	0.5
	85°C	3.3	2.5	1.9	1.0

Results And Discussion

This work aimed to develop an efficient optimized method for the determination of acetyl chloride content in various drug substances. Acetyl chloride is a volatile compound having boiling point 52°C and has no chromophores. For analysis of such a ultra-violet (UV) inactive volatile compounds gas chromatography (GC) is a suitable technology. Initially, we are trials were performed in a GC FID detector. In both techniques, in auto liquid and headspace sampler, the response of AC was very low. After lot of trails, GC-MS technique has been chosen. In this GC-MS, AC peak response was observed at higher levels and peak is not detected at specification level in scan mode. It is evident that mass spectroscopy detectors including electron impact (EI) or chemical ionization (CI) operating in the SIM mode, offer more sensitive and selective detection (compound specific) than most of the GC methods.

Trial experiments were conducted to select the suitable solvents for derivatization using methanol, ethanol and isopropyl alcohol and hence isopropyl alcohol selected as derivatization solvent. In addition, isopropyl alcohol is especially suitable for derivating acetyl chloride and is converted to Isopropyl acetate. Diluent prepared as Isopropyl alcohol and 1-Methyl-2-pyrrolidinone in the ratio of 1:9 v/v. By using a DB-1 capillary column with a dimension of 30 m x 0.32 mm ID x 3.0 µm film thickness. Due to its non-polar stationary phase (i.e. 100% dimethyl polysiloxane), this column has been chosen. In this column

AC peak was tailing observed in both techniques in auto liquid sampler and headspace sampler. We made few trials by changing different diluents and chromatographic conditions in GC-MS. But in these all trails not able to achieve required levels. Finally based on the tendency of reactivity of the AC, there is a possibility to develop a headspace derivatization method by GC-MS. Finally, overcome all the issues and selected Isopropyl alcohol as a derivatization solvent. The final development succeeded in DB-624 capillary column with a dimension of 30 m x 0.32 mm ID x 1.8 µm film thickness. Elution of AC was investigated using helium as carrier gas, with the constant flow 2.0 mL/min and keeping the column oven temperature initially 40°C is maintained for 4 minutes and then increased to 220°C at a rate of 20°C/minutes, followed by holding at 220°C for 7 minutes. The GC-MS spectrums of AC and derivatized peak i.e. isopropyl acetate were shown in Figs. 2a and b. The quantification and qualifier ions were selected for m/z-61 and m/z-87, respectively. Finally, the optimized method was validated as per International Council for harmonisation (ICH) guidelines.[43] The validation protocols such as accuracy, precision, linearity, specifcity, sensitivity and robustness were observed to be within the acceptance limit. This developed method was found to be linear with a correlation coefficient is greater than 0.999 for all four drug substances. The average recoveries of AC in BMH, IBR, TFD and ABM were obtained 106.1, 109.0, 97.4 and 101.6%, respectively. Finally, a new novel optimized method was developed and validated, with better peak shape and satisfactory separation was achieved on chromatographic conditions. A reliable and sensitive validated HS-GC-MS method for the determination of acetyl chloride as isopropyl acetate in various anti-cancer drug substances like bendamustine HCl, ibrutinib, trifluridine and asbemaciclib drug substances is presented. Based on validation data, it is concluded that method is specific, sensitive, linear, precise, accurate and suitable. Hence the validated HS-GC-MS method can be employed in to the routine analysis for the determination of acetyl chloride as isopropyl acetate in bendamustine HCl, ibrutinib, trifluridine and abemaciclib drug substances.

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References

1. Neil O, Maryadele J, The Merck index: an encyclopedia of chemicals, drugs, and biologicals. Merck Research Laboratories, Whitehouse Station, NJ, Merck, 2006.
2. Ribosepharm GmbH. Bendamustine product monograph. München, Germany, 2000.
3. Kath R, Blumenstengel K, Fricke HJ, Höffken K. Bendamustine monotherapy in advanced and re-fractory chronic lymphocytic leukemia. J Cancer Res Clin Oncol, 2001; 127(1): 48-54.
4. Bagchi S. Bendamustine for Advanced Sarcoma. Lancet Oncol 2007; 8(8): 674-679.
5. Friedberg, JW, Cohen P, Chen L, Robinson KS, Forero-Torres A, La Casce AS, et al. Bendamustine in patients with rituximab-refractory indolent and transformed non-Hodgkin's lymphoma: results from a phaseII multicenter, single-agent study;Journal of Clinical Oncology, 2008; 26(2): 204-210.
6. Cives M, Ciavarella S, Rizzo FM, De Matteo M, Dammacco F, Silvestris F. Bendamustine overcomes resistance to melphalan in mye- loma cell lines by inducing cell death through mitotic catastrophe; Cellular Signalling, (2013); 25(5): 1108-1117.
7. http://www.ncbi.nlm.nih.gov/pubmedhealth
8. http://www.cancer.gov/cancertopics/druginfo/fda-bendamustine-hydrochloride-indications
9. Pencheva I, Bogomilova A, Koseva N, Obreshkova D, Troev K. HPLC study on the stability of Bendamustine hydrochloride immobilized onto polyphosphoesters. Journal of Pharmaceutical and Biomedical Analysis, 2008; 48(4): 1143-1150.
10. Leoni LM, Bailey B, Reifert J, Bendall HH, Zeller RW, Corbeil J, Elliott G, Niemeyer CC. Bendamustine (Treanda) displays a distinct pattern of cytotoxicity and unique mechanistic features compared with other alkylating agents. Clin Cancer Res. 2008 January 1;14(1):309-17. doi: 10.1158/1078-0432.CCR-07-1061.
11. Pan Z, Scheerens H, Li SJ, Schultz BE, Sprengeler PA, Burrill LC, Mendonca RV, Sweeney MD, Scott KC, Grothaus PG, Jeffery DA, Spoerke JM, Honigberg LA, Young PR, Dalrymple SA, Palmer JT. Discovery of selective irreversible inhibitors for Bruton's tyrosine kinase. ChemMedChem. 2007 Jan;2(1):58-61. doi: 10.1002/cmdc.200600221.
12. US patent 7514444, Honigberg L, Verner E, Pan Z, Inhibitors of bruton's Tyrosine Kinase, pharmacyclics Inc April 7 2009.
13. Cameron F, Sanford M, Ibrutinib: First global approval, Drugs 2014; 74: 263-271.
14. Singh J, Petter RC, Kluge AF, Targeted covalent drugs of the kinase family, Curr. Opin. Chem. Biol. 2010; 14: 475-480
15. FDA approves Imbruvica for rare blood cancer, United States Food and Drug Administration, 2019.
16. Kim ES, Dhillon S. Ibrutinib: a review of its use in patients with mantle cell lymphoma or chronic lymphocytic leukaemia, Drug Pub Med 2015; 75(7):769-776.
17. Bagcchi, S. Ibrutinib in pretreated Waldenström's macroglobulinaemia. The Lancet Oncology, 2015; 16(5): e204.
18. News Release: FDA Expands Approved Use of Imbruvica for Rare Form of Non- Hodgkin Lymphoma, FDA, 2015; January 29.
19. Scheers E, Leclercq L, de Jong J, Bode N, Bockx M, Laene A, Cuyckens F, Skee D, Murphy J, Sukbuntherng J, Mannens G. Absorption, metabolism, and excretion of oral ^{14}C radiolabeled ibrutinib: an open-label, phase I, single-dose study in healthy men Drug Metab. Dispos. Pubmed., 2015; Feb: 43(2): 289-97.
20. https://www.drugbank.ca/drugs/DB00432
21. https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/207981s000lbl.pdf
22. Kish T, Uppal P. Trifluridine/ Tipiracil (Lonsuf) for the treatment of metastatic colorectal cancer. Pharmacy and Therapeitics. 2016; 41(5): 314-317, 325.
23. Hong DS, Abbruzzese JL, Bogaard K, Lassere Y, Fukushima M, Mita A, Kuwara K and Hoff PM. Phase I study to determine the safety and pharmacokinetics of oral administration of TAS – 102 in patients with solid tumors. Cancer. 2006; 107(6): 1383-90.
24. Temmink OH, Prins HJ, van Gelderorp E, Peters GJ. The Hollow Fibre assay as a model for in vivo pharmacodynamics of fluoropyrimidines in colon cancer cells. 2007; Jan 15; 96 (1): 61-6.
25. Tanaka N, Sakamoto K, Okabe H, Fujioka A, Yamamura K, Nakagawa F, Nagase H, Yokogawa T, Oguchi K, Ishida K, Osada A, Kazuno H, Yamada Y, Matsuo K. Repeated oral dosing of TAS – 102 confers