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International Journal of Current Research
Vol. 3, Issue, 11, pp.400-404, October, 2011

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

DETERMINATION OF METHYL BROMIDE AT SUB PPM LEVELS IN FINISHED PHARMACEUTICAL PRODUCT BY GAS CHROMATOGRAPHY

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ARTICLE INFO

Article History:
Received 7th June, 2011
Received in revised form
5th August, 2011
Accepted 9th September, 2011
Published online 30th October, 2011

Key words:
Methyl bromide,
Isopropyl bromide,
Gas chromatography,
Electron capture detector,
Validation.

ABSTRACT

Methyl Bromide is a volatile organohalide well known for its genotoxic properties. Due to its genotoxic nature, health authorities (regulatory bodies) across the globe require that it be kept well within stringent limits in active pharmaceutical ingredients and finished pharmaceutical products. A variety of techniques like HPLC with derivatization, GC with mass detection and GC with atomic emission detection have been employed in the past as well as is currently in use by researchers across the globe for quantitative determination of Methyl Bromide. Although several methods are available in various literatures it may not be feasible to use those methods due to the requirement of highly sophisticated instruments like Mass Detector or due to high cost of analysis or due to poor reproducibility and cumbersome methodology. A sensitive and accurate direct gas chromatographic method was developed using Electron Capture Detector (ECD). In this method Methyl Bromide was well separated from Isopropyl Bromide. The detection limit and quantitation limit were established at 0.015 and 0.0375 ppm respectively during the successful validation of the method. The proposed method was found to be Precise, Specific, Linear, Rugged and Robust.

INTRODUCTION

Clopidrogrel (Methyl (S)-alpha- (2-Chlorophenyl)- 6,7-dihydrothieno [3,2-C] pyridine-5 (4H)-acetate) salt is a potent platelet anti-aggregation prodrug that is very similar to Ticlopidine in its structure and mechanism of action. It is used to prevent heart attacks and strokes in persons with heart disease like unstable angina, recent heart attacks or blood circulation disease. Clopidrogrel exhibits its antiplatelet aggregating property by inhibition of binding of ADP to low affinity receptors on the platelet cell membrane. The drug specifically and irreversibly inhibits the P2Y12 subtype of ADP receptor, which is important in aggregation of platelets and cross-linking by the protein fibrin [1] The blockade of this receptor inhibits platelet aggregation by blocking activation of the glycoprotein IIb/IIIa pathway. The IIb/IIIa complex functions as a receptor mainly for fibrinogen and vitronectin but also for fibronectin and von Willebrand factor. Activation of this receptor complex is the "final common pathway" for platelet aggregation and is important in the cross-linking of platelets by fibrin. In free base form, Clopidogrel is relatively unstable under increased moisture and temperature, accordingly acid addition salts of Clopidogrel, which are more stable solids and simpler for purification were developed like hydrochloride, hydrobromide, hydrogen sulfate and taurocholate [2,3]. Clopidogrel hydrogen sulfate (bisulfate) is the commercial available salt, marketed as Plavix tablets by Bristol-Myers Squibb and Sanofi Aventis. This work however, deals with Clopidogrel Hydrobromide salt which comparatively exhibits better solubility and chemical stability characteristics. Strong acid/alcohol interactions during the process of drug salt formation produce alkylating agents such as alkyl halides and alkyl esters. Alkyl halides are categorized as genotoxic impurities (GTI) based on structure-activity-relationship. During the manufacturing process of Clopidrogrel hydrobromide, formation of methyl bromide is possible due to residual methanol available in the manufacturing process and may also be formed due to thermal interaction in presence of methanol. This paper discusses analytical methodology for a specific class of GTI, the alkyl halides. GTI's are unusually toxic material which could potentially impact genetic material by mutation. These changes to the genetic material, caused by exposure to very low levels of a genotoxin, can lead to cancer. While ICH published adequate controls for general process-related impurities (Q3A, Q3B), Pharmaceutical Genotoxic impurities (GTI) gained global prominence when regulatory bodies like European Medicines Agency (EMEA) and United States Food and Drug Administration (USFDA) [4,5] some few years back published stringent qualification thresholds lower than the default values set by ICH guidelines. These dictums confirmed that the existing ICH thresholds may not be acceptable for such DNA-reactive genotoxins or carcinogenic impurities. Genesis of these impurities could be from variety of sources, namely but not limited to starting materials, reagents,

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active pharmaceutical ingredient (API) synthetic process that get carried over into the final product. In addition, the API itself can decompose to form genotoxic impurities or they can form in the drug product by reaction between excipients or containers and the API. In some cases, trace level of solvents could react with intermediates and form potential genotoxic impurities. Determination of methyl bromide at trace levels requires highly sensitive analytical methodology. A variety of techniques like HPLC with derivatization, GC with MS detection and GC with AED have been employed in the past and are currently used by researchers for quantitation of Methyl Bromide. In certain cases it may not be possible to employ these techniques either due to the requirement of highly sophisticated analytical instrument or due to high cost of analysis or due to poor reproducibility and cumbersome methods. Based on above reasons, industry recognized the need to develop sensitive analytical technique that was simple, cost effective and that employed commonly available instruments and that had the capability to quantitate Methyl Bromide at nanogram levels

EMEA proposes the use of a "threshold of toxicological concern" (TTC) for GTIs where TTC refers to an exposure level to compounds that does not pose a significant risk (one in 10,000 lifetime risk) for carcinogenic effects. As there are no compound-specific safety data, EMEA recommends a generic safe dose of 1.5 µg/patient/day for controlling levels of genotoxic impurities in drug substances (and drug products), this can be considered as an acceptable qualification threshold for supporting a marketing authorization application by EMEA and US FDA. A TTC of 1.5 µ g/day for a given day with a dose of 1.0 g/day would require an analytical capability of achieving a testing limit of 1.5 ppm. Ideally, conventional analytical instrumentations in pharmaceutical analysis such as HPLC with UV detection (for typical non-volatile analytes) or GC with FID detection (for volatile small molecules), should be employed as the standard first attempt for GTI analysis, but are often inadequate for accurate determination of analytes at low ppm levels, depending on properties of the analytes and sample matrices. Consequently, in the past few years, analytical scientists in the pharmaceutical industry have strived to develop analytical strategies to meet this challenge. A recent review article on the advances made in the testing of different structural classes of GTI makes an attempt to provide analytical guidance to pharmaceutical industry [6]. General methods for the determination of alkyl halides have been discussed using different techniques including electron capture detector [7, 8, 9]. But discussions are general and not specific to the methyl bromide. Although the USEPA 524.2 method is available for the analysis of volatile organic compounds and is suitable for analysis of Methyl Bromide but it has a limitation that it requires a MS detector that makes the method more costly and sometimes it is not feasible due to non availability of MS. Similarly certain direct HPLC methods are available but with the limitation that the detection wavelength is very low (Lambda max of methyl bromide 202nm). In other cases the use of derivatization makes the technique cumbersome and complicated along with decrease in sensitivity and reproducibility. Even though several methods were reported in the literature for the quantification of methyl bromide, to the best of our knowledge, none of the methods are practically workable in quantifying methyl bromide from Clopidrogrel present work focuses on the detection and quantification of Methyl Bromide in finished dosage form. A novel method using GC with electron capture detector was developed and validated to determine Methyl Bromide in Clopidrogrel hydrobromide drug substance and drug product. The analytical method is specific and is capable of simultaneously quantifying other alkyl bromides like Ethyl Bromide and Isopropyl Bromide in presence of solvents like methanol, isopropyl alcohol, and dichloromethane.

MATERIALS AND METHODS

Chemicals and reagents: HPLC grade toluene was purchased from Merck (Merck Specialties Private limited, Mumbai, India), Methyl bromide standard ~ 25% in toluene and Ethyl bromide 98 % were purchased from Spectrochem Private Limited (Mumbai, India). Isopropyl bromide was purchased from Fluka and isopropyl alcohol, dichloromethane and methanol were purchased from Merck specialties Private Limited (Mumbai, India). Clopidrogrel tablets were provided by Matrix Laboratories, R&D.

Instruments: The GC system consisted of an Agilent Gas chromatograph system 6890N Network system (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a built-in Electron capture detector, auto sampler (7683B). Data collection and analysis were performed using and Empower II software (Waters Corporation, Milford, MA).

Chromatographic conditions: Separation was achieved on a DB-1 GC capillary column, 30 meters length, 0.32 mm ID and 3 µm film thicknesses, fused silica capillary column. (Agilent Technologies, Palo Alto, CA, USA). The initial oven temperature of 35°C maintained for 8 minutes was raised to 240°C at the rate of 20°C and held for 6 minutes. Injector temperature and detector temperature were 150 and 300°C respectively. Helium (carrier) and nitrogen (make up) were used at 1.5 and 30 mL/min respectively. Injector was used in split mode with split ratio of 1:10. Injection volume used was 2.0 µ L. Toluene was used as diluent. The sample was chromatographed for 25 minutes

Standard and Sample Preparation: Standard solution of methyl bromide in toluene was prepared using 25% methyl bromide in toluene at a concentration of 0.375 ppm. The relative standard deviation for the methyl bromide peak from six replicate injections was evaluated as part of system suitability with the acceptance criteria of not more than 5.0%. In case of sample preparation, tablet equivalent to 1500 mg of Clopidrogrel was taken in 20 mL of toluene. The clear solution was used for the analysis of methyl bromide content.

RESULTS

Method Development: As a part of development, experiments were performed using gas chromatography with flame ionization detector to achieve a minimum sensitivity of 0.0375 ppm Methyl Bromide. But due to the inherent lack of sensitivity, limit of quantitation was not achieved beyond 0.19 ppm. This led us to explore electron capture detector that is well known for its higher sensitivity. We selected Electron Capture Detector due to its specificity for electronegative

increase in sensitivity of the method by about 10 times which was evident by reduction of quantitation limit by ten folds. A limit of quantitation as low as 0.0375 ppm for methyl bromide with good repeatability as evidenced by low relative standard deviation (RSD=2.6) calculated for area counts of six injections of standard solution was obtained. This method was subjected to validation to ascertain its suitability for the intended use.

Table 1: Linearity data for Methyl Bromide

Linearity data for methyl bromide		
Serial #	Concentration (µg/mL)	Mean Area
1	0.038	75.0114
2	0.19	258.3393
3	0.304	399.0342
4	0.38	479.8458
5	0.76	906.3887
6	1.899	2073.5039

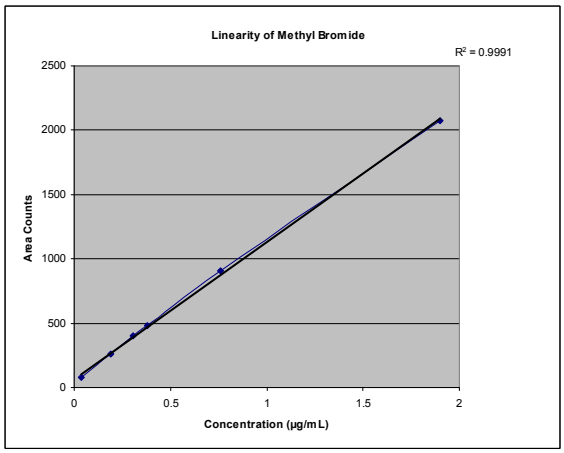


Figure 1: Linearity of methyl bromide

Table 2: Summary of Regression Parameters

Summary of Regression Parameters			
Serial #	Parameter	Obtained Value	Acceptance Criteria
1	Correlation Coefficient	0.999	The Correlation Coefficient should not be less than 0.990
2	Slope	1066.0956	
3	Y-Intercept	64.1827	
4	Residual Sum of Squares	2431.805127	

specificity. Methyl Bromide eluted at about 4.1 minutes while Ethyl Bromide eluted at about 7.1 minutes and Isopropyl Bromide eluted at about 9.8 minutes indicating that the method has good resolving capability for these analytes. The GC chromatogram is shown in Figure 3.

Table 4: Intermediate Precision

SERIAL #	SET # 1	SET # 2
1	0.93	0.98
2	0.92	0.97
3	0.91	0.99
4	0.92	0.98
5	0.94	0.96
6	0.93	0.96
RSD	1.13	1.24
Overall RSD	2.89	

Set # 1 = analyst 1, column 1, system 1 and day 1; Set # 2 = analyst 2, column 2, system 2 and day 2.Acceptance Criteria: Overall RSD should be not more than 10.0 %

Repeatability: Methyl Bromide was prepared at 0.38 ppm absolute and injected in six replicates. The RSD (n=6) values obtained for the area of Methyl Bromide is 0.7 %, indicating a high degree of repeatability in the method.

Linearity: Linearity parameter was ascertained by measuring area counts for Methyl Bromide over the range of 0.0375ppm to 1.88 ppm absolute concentration. Each preparation (n=6) was injected and the area calculated was plotted against the concentration. The squared regression coefficients obtained for methyl bromide is 0.999. The results revealed an excellent linearity.

Accuracy: Accuracy was established through recovery experiments by spiking known amount of Methyl Bromide at 2.5, 5 and 25 ppm levels with respect to sample concentration. Triplicate preparations were made at each level and chromatographed. Recovery was found to be between 87.6 and 100.6 % with the percentage relative standard deviation of less than 5 %. Data shown in Table 3 demonstrate the accuracy of the method

Detection and quantitation limit: The detection limit (DL) and quantitation limit (QL) were determined for Methyl Bromide by using signal to noise (S/N) ratio method. The minimum concentration at 3:1 S/N ratio was established as DL of the method, and the concentration at 10:1 S/N ratio was established as QL of the method. The DL and QL were 0.015 and 0.0375 ppm respectively. The repeatability at QL was

Table 3: Results of recovery studies for methyl bromide for clopidrogrel 300 mg tablets

Preparation No.	Recovery Level	Amount Added (PPM)	Amount recovered (PPM)	% Recovery	Mean % Recovery	%RSD
1	50%	2.5	2.514	100.6	97.9	4.7
2	50%	2.5	2.514	100.6		
3	50%	2.5	2.314	92.6		
1	100%	5.0	4.614	92.3	91.6	1.3
2	100%	5.0	4.614	90.3		
3	100%	5.0	4.614	92.3		
1	500%	25.6	22.414	87.6	89.2	2.9
2	500%	25.6	22.514	87.9		
3	500%	25.6	23.614	92.2		
Acceptance Criteria		a. The individual % recovery at each level should be between 85.0 % and 115.0 % b. The average % recovery at each level should be between 85.0 % and 115.0 % c. The RSD for each level should be not more than 6.0 %				

Method Validation

Specificity: Interference of Ethyl Bromide and Isopropyl

injections. This indicates that the proposed method is highly

Bromide

Table 5: Methyl Bromide-Robustness (Flow Variation)

System Suitability Parameter	Observed value with Flow Rate			
RSD	1.35 mL/min	(Flow Minus)	1.5 mL/min (Control)	1.65 mL/min (Flow Plus)
	3.8		2.9	3.4
Acceptance Criteria	The relative standard deviation of area counts for Methyl Bromide peak obtained from six replicate injections of standard solution should be not more than 5.0%			

Table 6: Methyl Bromide-Robustness (Column Oven Temperature Variation

System Suitability Parameter	Observed value with Flow Rate					
RSD	30°C	(Temperature Minus)	35°C	(Control)	40°C	(Temperature Plus)
	0.9		3.4		1.3	
Acceptance Criteria	The relative standard deviation of area counts for Methyl Bromide peak obtained from six replicate injections of standard solution should be not more than 5.0%					

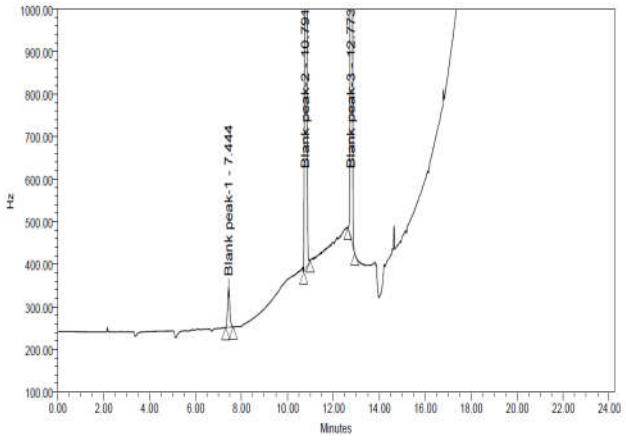


Fig. 2: Blank Chromatogram

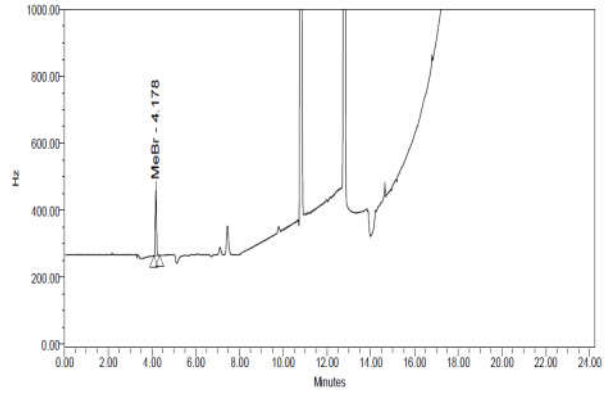
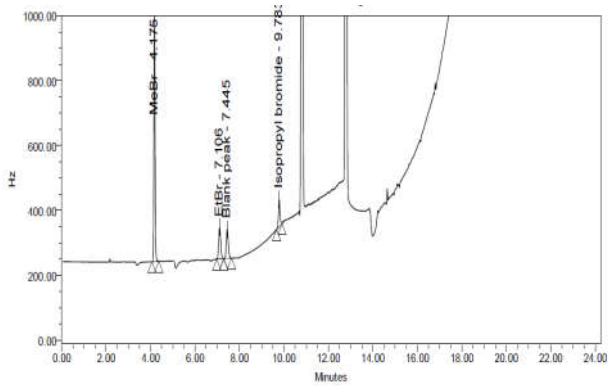


Fig. 3: Standard Chromatogram-Methyl Bromide



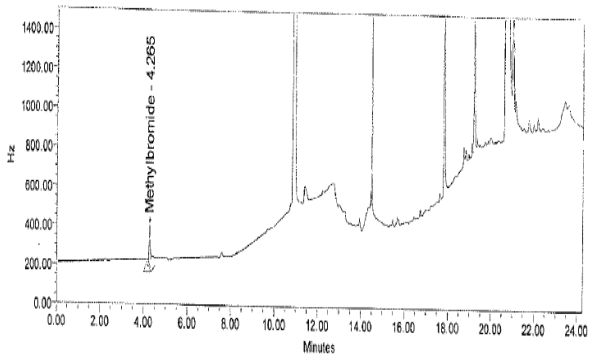


Fig. 5: Chromatogram of Clopidrogrel tablets 75 mg

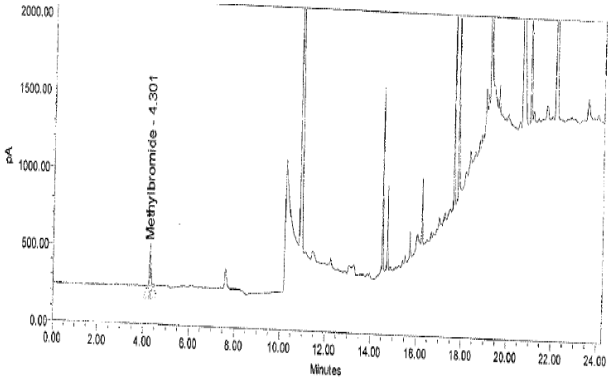
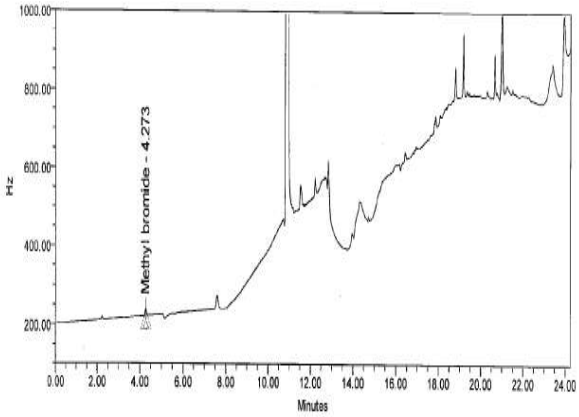


Fig. 6 : Chromatogram of Clopidrogrel tablets 300 mg



sensitive and reproducible. QL Chromatogram is shown in Figure 7

Intermediate Precision: Ruggedness of the method was evaluated by performing the sample analysis in six replicates using two different columns, different GC instruments and different analyst on different days and the results are summarized in Table 4. The RSD values of less than 3.5% for Methyl Bromide content indicate that the method adopted is rugged.

Robustness: This study was performed by making small but deliberate variations in the method parameters. The effect of variations in flow rate of carrier gas and column oven temperature was studied and the results are presented in Table 5 and Table 6 respectively. Under all the variations, system suitability requirement is found to be with in the acceptance criteria and hence the proposed method is robust.

DISCUSSION

Avoidance of potential genotoxic impurities through drug substance synthesis process may be impractical in many instances, present at trace levels, organohalides due to their reactive nature, are invariably unstable posing a major challenge to the analytical chemist in terms of recovery, reproducibility and sensitivity. The simple GC method described in this research paper for Methyl Bromide in finished pharmaceutical product obviates the need to derivatize the sample to enhance detectability. The method can be easily transferred from R&D lab to manufacturing quality control site since it does not involve complex sample preparation or very sophisticated analytical setup. This robust method demonstrated excellent linearity, specificity, precision, sensitivity and accuracy which can be routinely used for the quantification of Methyl Bromide in Clopidogrel Hydrobromide Tablets. Though the authors have not checked the substrate interference, going by the methodology they are certain that this method can be extended to other different pharmaceutical products. Additionally this method demonstrated the capability to separate methyl bromide from ethyl bromide and isopropyl bromide with resolution greater than 12.

Acknowledgement

The authors thank Matrix Laboratories Limited for providing the required support and resources.

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