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Development and validation of GC-MS method for analysis of chloropyramine hydrochloride in ointments

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Abstract: *A simple, rapid, sensitive, specific, accurate, and reproducible method for the determination of chloropyramine hydrochloride in ointments based on gas chromatography – mass spectrometry detection was developed and validated. Dissolution of chloropyramine hydrochloride in the ointment was performed with 0.5 M HCl. After alkalization with 25% (V/V) NH_4OH, chloropyramine base was extracted with chloroform. The method was validated in respect of linearity, specificity, precision, recovery, limit of detection (LOD), limit of quantification (LOQ) and stability. In terms of performances: Recovery 90.0 – 98.7%, LOD 0.04 mg/g, LOQ 0.132 mg/g, specificity, selectivity and precision the proposed method was found suitable for routine analysis.*

Keywords: *chloropyramine hydrochloride, chloropyramine base, gas chromatography- mass detection, ointment*

I. INTRODUCTION

Chloropyramine is a first generation antihistamine drug approved in some Eastern European countries for the treatment of allergic conjunctivitis, allergic rhinitis, bronchial asthma, and other allergic conditions [1]. Chloropyramine is known as a competitive reversible H1-receptor antagonist. By blocking the effects of histamine, the drug inhibits the vasodilatation, increased vascular permeability, and tissue edema associated with histamine release in the tissue [2]. The use of topical antihistamines preparations represented a major advance in dermatology. Chloropyramine hydrochloride (N-[(4-chlorophenyl)methyl]-N',N'-dimethyl-N-pyridin-2-ylethane-1,2-diamine hydrochloride) is an antihistamine of ethylenediamine group (Figure 1) indicated for the treatment of several pathologies due to its anti-allergic and anti-inflammatory effects [3, 4]. This antihistamine is frequently incorporated in ointments and creams [5].

Ointments are homogeneous, viscous, semi-solid preparation, most commonly greasy, thick oil (oil 80% - water 20%) with a high viscosity that is intended for external application to the skin or mucous membranes. Ointments have a water number that defines the maximum amount of water that it can contain. They are used as emollients or for the application of active ingredients to the skin for protective, therapeutic, or prophylactic purposes and where a degree of occlusion is desired [6]. The vehicle of an ointment is known as the ointment base. Commonly they are molecules self-assembled in water or in oil, leading to the formation of a well defined microstructure. These heterogeneous systems can interfere with drug separation and detection, and an adequate analytical method is needed to analyze the drug carried by these systems.

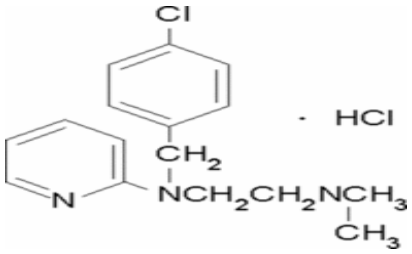

Figure 1. Chemical structure of chloropyramine hydrochloride

Gravimetric and spectrophotometric methods have been described for the simultaneous determination of chloropyramine, amitriptiline, imipramine and antazoline in coated tablets [7]. A novel High - performance liquid chromatography method (HPLC) for the simultaneous determination of antihistamine bamipine, sympathomimetic amines and dextromethorphan in bulk drug material have been developed [8]. HPLC method was also reported for the determination of antihistamines pheniramine and pyrilamine in two over the counter cold medications [9]. HPLC remains the analytical method of choice, especially for analysis of the topical formulations, owing to their complex composition [10].

In the last two decades a remarkable advancement in the hyphenated techniques and its application in pharmaceutical analysis have been achieved. A variety of hyphenated techniques such as liquid chromatography tandem mass spectrometry (LC-MS), gas chromatography with mass detection (GC-MS) etc. have been applied in the analysis of pharmaceuticals [11]. However, up to our knowledge, no GC-MS method for the analysis of chloropyramine hydrochloride in ointments has been described. The aim of this study was to develop a simple, rapid, specific, precise and accurate method based on GC-MS technique for the determination of chloropyramine hydrochloride in ointments. The method was validated in respect of linearity, specificity, precision, recovery, limit of detection (LOD), limit of quantification (LOQ) and stability [12, 13].

II. MATERIALS AND METHODS

2.1. Instrumentation and chromatographic conditions

The method was performed on a Shimadzu GC-MS model QP 2010 Ultra. Chromatographic separation was achieved on a fused silica ZB-5 capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness), supplied by Phenomenex (Torrance, USA). Helium purity 99.99999% was used as carrier gas. Operating conditions are given in Table 1.

Table 1. GC-MS operating conditions

Column oven T		180 ^{0}C	
Injection T		250 ^{0}C	
Injection mode		Splitless	
Sampling time		1 min	
Flow control mode		Pressure	
Pressure		60 kPa	
Column flow		0.62 mL/min	
Linera velocity		30.2 cm/sec	
Purge flow		6.0 ml/min	
High pressure injection		On	
High pressure injection pressure		250 kPa	
High pressure injection time		1.0 min	
Oven temperature program			
Rate	T(^{0}C)		Hold time (min)
-	180.0		0
5.00	240		15
Injector type		PTV	
PTV carrier		Yes	
PTV purge		Yes	
Equilibrium time		3.0 min	
Ion source temperature		200 ^{0}C	
Interface temperature		250 ^{0}C	
Solvent cut time		1.0 min	
Detector gain mode		Relative	
Detector gain		0.2 kV	
MS table			
Start time		11.92 min	
End time		13.92 min	
Acquisition mode		Selected ion monitoring (SIM)	
Event time		0.3 sec	
Ch1-m/z		58	
Ch2-m/z		125	
Ch3-m/z		71.0	

2.2. Reagents and standards

Chloropyramine hydrochloride purity ≥99.8% was obtained from Fluka, Germany Chloropyramine-base purity ≥99.7% was obtained from Alkaloid, Macedonia. The ointment was composed of white wax (pellets - Sigma Aldrich, Germany) and white petrolatum (Sigma Aldrich, Germany). Chloroform (Merck, Germany) gas chromatography grade was used for the extraction and preparation of standard solutions. Ammonium hydroxide (25%, V/V) was obtained from Fluka, Germany. Hydrochloric acid (37%, V/V) was obtained from Sigma Aldrich, Germany. Anhydrous sodium sulfate was obtained from Merck, Germany. Water was obtained by distillation.

2.3. Preparation of the ointment

All components, the white wax and the petrolatum, were accurately weighed. The white wax was melted on a hot plate at 70 – 75^0 C. When the wax was completely melted, the petrolatum was added and the entire mixture was put on a hot plate until liquefied. Following liquefaction, the mixture was removed from heat and was allowed to congeal. The mixture was stirred until it begun to congeal. Drug-loaded ointments were prepared by adding 1% (W/W) chloropyramine hydrochloride to the ointment at room temperature. The mixture was gently shaken to ensure complete mixing and dissolution. The drug-containing ointments and solutions were shielded from light by storing in flasks wrapped with aluminum foil.

2.4. Preparation of standard solutions

Chloropyramine standard stock solution with the concentration of 1mg/mL (i) was prepared in chloroform by transferring 10 mg of chloropyramine standard accurately weighted to a 10 mL volumetric flask and filled up with chloroform to the mark. An aliquot of 0.25 mL of the standard stock solution (i) was transferred to a 25 mL volumetric flask and was diluted with chloroform to obtain the concentration of 40 µg/mL (standard stock solution ii).

Working standard solutions, in a concentration range of 0.4 – 4.0 µg/mL, were prepared by dilution of the standard stock solution (ii) with chloroform. 1 µL of each concentration was injected into the GC-MS system and the area under curve (AUC) for each peak was plotted versus chloropyramine concentration. The analyses were carried out in triplicate and a straight line standard curve was obtained by linear regression of the experimental data.

2.5. Sample preparation

An aliquot of 0.1 g of homogenized ointment which contains 1% (W/W) chloropyramine hydrochloride was accurately weighed and dissolved in approximately 10 mL 0.5 M HCl. The solution was quantitatively transferred into the 100 mL separatory funnel. pH value of the solution was adjusted to 9-10 with 25% NH_4OH (V/V). After alkalization chloropyramine-base was extracted with 40 mL chloroform. Chloroform layer was transferred through anhydrous sodium sulfate into the 50 mL volumetric flask and was filled with chloroform up to the mark. 1 mL of the solution was transferred into the 10 mL volumetric flask. The solution was filtered through a 0.22 µm Millipore membrane filter. 2 mL of the filtrate were transferred into the auto - sampler vial. 1 µL were injected into the injector port of the GC-MS. All the determinations were conducted in triplicate.

2.6. Determination

Determination of chloropyramine was performed by GC-MS in SIM mode under the operating instrumental conditions shown in Table 1. Quantification was made by comparison of area under the curve (AUC) obtained for chloropyramine extract with AUC obtained for the corresponding analytical standards.

2.7. Validation of the method

The method was validated in accordance with International Conference on Harmonization guidelines (ICH-2003) for validation of analytical procedures [12].

Specificity and Selectivity

These parameters were determined by comparing the chromatograms of the chloroporymanine standards with the chromatograms obtained for drug-loaded ointment and ointment without drug.

Linearity

The linearity was determined from the analytical curves obtained by GC-MS analysis of chloropyramine standard solutions.

Recovery experiment

Recovery was determined by the standard addition method. The ointment samples in which 2.5mg/g of the chloropyramine hydrochloride had been incorporated previously were fortified with different amounts of chloropyramine hydrochloride solution prepared in 0.5 M HCl. The final concentrations of the fortified samples were 5 mg/g, 7.5 mg/g, 10 mg/g, 12.5 and 15 mg/g, respectively. The recovery experiments were performed in triplicate for each concentration.

Precision

The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). The intra-day precision was calculated as relative standard deviation (RSD) of results from ten standard samples, during the same day, and the inter-day precision was studied by comparing the assays on two different days. Ten samples of ointment fortified with chloropyramine hydrochloride at 10 mg/g were prepared and assayed, and the standard deviation (SD) and RSD were calculated.

Calculation of LOD and LOQ

LOD and the limit of quantification LOQ were calculated according to the formulas LOD = 3.3 · SD/slope and LOQ = 10 · SD/slope [14].

III. RESULTS AND DISCUSSION

3.1. Method optimization

Because of poor solubility of chloropyramine hydrochloride (salt) in water and in the majority of organic solvents (polar and non-polar), in our experiment as a suitable solvent for chloropyramine hydrochloride dissolution we used 0.5 M HCl. In their study, Iliaszenko *et al.* used 0.5 M HCl for dissolution of several tertiary amine hydrochloride drugs in coated tablets, prior their spectrophotometric determination [7].

Chloropyramine base was deliberated from the salt by addition of 25% NH_4OH (V/V) to pH 9-10. Extraction of the free base was efficiently performed with chloroform.

In order to improve specificity and selectivity and minimize interferences from ointment or solvent systems that may occur in spetrophotometric or HPLC determinations, we performed the analysis using GC-MS system. Selectivity was obtained by operating in selected ion monitoring (SIM) mode with target ion (58 m/z) and reference ions (71 m/z, 125 m/z). Figure 1 depicts the mass spectrum of chloropyramine hydrochloride.

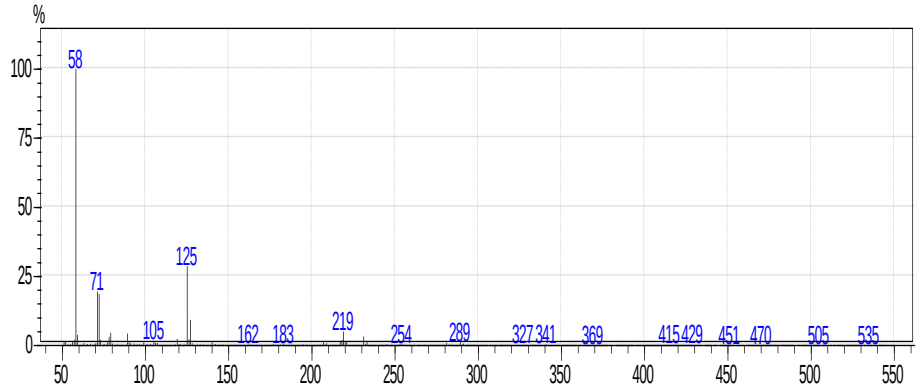

Figure 2. Mass spectrum of chloropyramine hydrochloride

3.2. Method validation

Recovery, Precision and Linearity

Statistical data for mean recovery, precision data and linearity of the method for the determination of chloropyramine hydrochloride in ointments are shown in Table 2.

Table 2. Statistical data for mean recovery, precision data and linearity of the method

Fortification level (mg/g)	FA found - mean value (mg/kg ± SD)	Recovery (%), n=3	RSD (%)	Regression equation
5.0	4.5 ± 0.12	90.0	2.67	y = 1.032x – 0.74 R^2 = 0.9996
7.5	6.9 ± 0.18	92.0	2.61	
10.0	9.6 ± 0.28	96.0	2.92	
12.5	12.1 ±0.34	96.8	2.81	
15.0	14.8 ± 0.32	98.7	2.16	

From the obtained results, it can be noticed that the proposed method is accurate and precise enough for the determination of chloropyramine hydrochloride in ointments. The obtained values for correlation coefficient R^2 = 0.9996 indicated that the method has a good linearity. High analytical recoveries ranging from 90% to 98.7% were obtained for chloropyramine hydrochloride determination.

Repeatability and Reproducibility

Statistical data for within day repeatability and between day reproducibility of the method for the determination of chloropyramine hydrochloride are shown in Table 3.

Table 3. Statistical data for repeatability and reproducibility of the method

t_R /min	Within day Repeatability (RSD, %); n=10		Between day Reproducibility (RSD, %); n=25	
12.941	0.112	3.27	0.190	4.28

The calculated RSD values for retention time (t_R) for the within day repeatability and between day reproducibility were found to be 0.112% and 0.19%, respectively which indicated good precision of the t_R. Good precision was also obtained for the within day repeatability and between day reproducibility of the peak area (RSD 3.27% - 4.28%).

Stability studies

Five aliquots of the extracts of quality control (QC) samples with the nominal concentration of 10mg/g chloropyramine hydrochloride were stored at -20^{0}C over the period of 12 weeks. It was found that extracts were stable within a period of 4 weeks. Figure 3 shows an overlay of chromatograms of extracts of chloropyramine base in chloroform over the period of 4, 8 and 12 weeks, respectively.

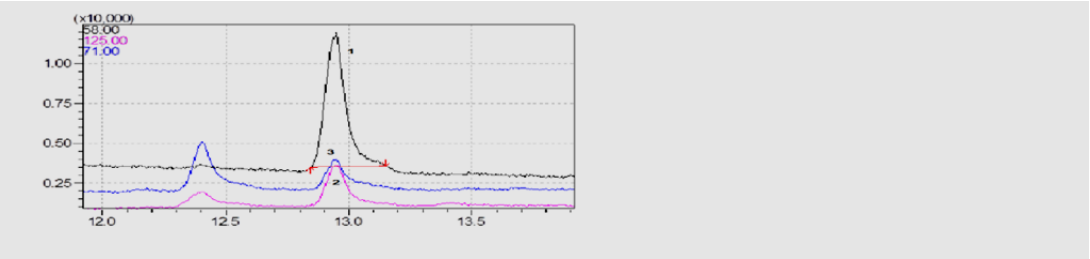

Figure 3. Overlay of chromatograms of extracts of chloropyramine base in chloroform (**1**-after 4 weeks, **2**- after 8 weeks and **3**-after 12 weeks of storage at -20^0 C).

With the proposed methods the calculated values for LOD and LOQ were 0.04 mg/g and 0.132 mg/g, respectively.

IV. CONCLUSION

In summary, the described method is rapid, sensitive, specific, accurate, and reproducible. It was successfully used for routine determinations of chloropyramine hydrochloride in commercially marketed ointments.

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