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DETERMINATION OF THE NASAL DECONGESTANT, OXYMETAZOLINE HYDROCHLORIDE, IN PHARMACEUTICAL FORMULATIONS BY HPLC

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ABSTRACT

A liquid chromatographic procedure for the analysis of oxymetazoline hydrochloride in pharmaceuticals utilizing reversed-phase ion-pairing was developed. Isolation of the analyte was carried out under isocratic conditions with a octadecylsilane column and an aqueous mobile phase containing methanol (70%), acetic acid (1%) and 0.005M sodium pentanesulfonate with detection at 280 ηm. The procedure was applied to a drug product quality survey including the bulk drug substance and twenty-one nasal decongestant formulations. The overall precision (CV) for the liquid product assays ranged from 0.11 to 2.02% and recovery values based on sample fortification ranged from 97.5 to 100.5%. The procedure is simple to perform and specific with respect to common formulation excipients.

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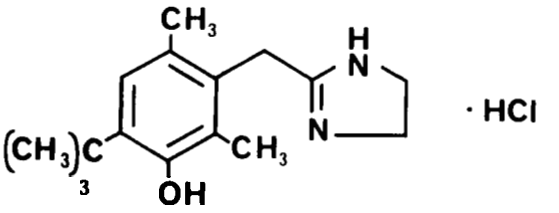

FIGURE 1

Structure of oxymetazoline hydrochloride

INTRODUCTION

Oxymetazoline, a derivative of imidazoline, is an adrenergic stimulant used primarily as a vasoconstrictor. Due to its longer-acting effects compared to other adrenomimetic amines such as epinephrine and lack of CNS depression, it has found wide usage as a nasal decongestant (1). The compound is administered as the hydrochloride salt (Fig. 1) usually at a concentration level of 0.05%.

Colorimetric assay procedures for the determination of oxymetazoline HCl in pharmaceutical dosage forms have found widespread popularity as evident by the number of reports in the literature. Sane et al (2) first reported a colorimetric assay procedure based on an acid-dye extraction using bromocresol green and bromophenol blue. Other colorimetric methods employing various dyes and color developing reagents have also been proposed (3-9). A two-phase titrimetric assay procedure applied to five imidazoline derivatives including oxymetazoline HCl in pharmaceuticals has been reported by Massaccesi (10). A gas-liquid chromatographic method for determining oxymetazoline HCl and three other imidazolines in pharmaceuticals has recently been described by the same author (11). Gas -liquid chromatography has also been used for the characterization of drugs of forensic interest including oxymetazoline for identification purposes (12). The application of high-performance liquid chromatography (HPLC) to the separation of oxymetazoline and other imidazoline derivatives of

pharmaceutical interest was first reported in 1973 by Mollica and co-workers (13). Chromatography was carried out using either a strong cation or a strong anion-exchange pellicular resin and buffered mobile phases containing 10% methanol. Retention data for qualitative purposes and including oxymetazoline has been compiled using normal phase (14), reversed-phase (15) and ion-pairing (16) HPLC. Palma et al (17) have demonstrated the use of a reversed-phase HPLC procedure employing an octadecylsilane column and methanol-0.05F phosphoric acid mobile phase for the analysis of oxymetazoline HCl in three commercial decongestant preparations. A comprehensive study of the chromatographic behavior of eight different imidazoline derivatives including oxymetazoline has recently been reported by DeSchutter and co-workers (18). This work involved the use of reversed-phase HPLC with ion-pairing and an amine modifier present in the mobile phase. The effects of the concentration of ion-pairing reagent, amine modifier, water and sodium chloride were studied in addition to pH and column temperature. Optimized chromatographic conditions were described for the determination of coumazoline and tetrahydrozoline in two commercial decongestant preparations.

This communication describes a rapid, precise and accurate reversed-phase ion-pairing HPLC procedure for the determination of oxymetazoline HCl as the bulk drug substance and in commercial formulations. The proposed method was developed for use with a drug product quality survey for this nasal decongestant.

EXPERIMENTAL

Reagents and Materials

Oxymetazoline hydrochloride was USP reference standard, Lot-G. Methanol (Burdick and Jackson Laboratories, Inc., Muskegon, MI) and 1-pentanesulfonic acid, sodium salt (Fisher Scientific Company, Fair Lawn, NJ) were HPLC grade. Acetic acid (Mallinckrodt, Inc., Paris, KY) was analytical reagent grade. All excipient materials used in synthetic formulations and other common ingredients and chemicals used were

USP or reagent grade. Pharmaceutical products employed in this study were obtained through various commercial sources. All liquid formulations were packaged in plastic containers. Distilled, deionized water passed through a 0.2 μm Versapor membrane filter (Gelman Sciences Inc., Ann Arbor, MI) was used throughout.

Chromatographic Instrumentation

The HPLC component system consisted of an Altex/Beckman Model 100A pump (Beckman Instruments, Inc., Berkeley, CA) equipped with a LDC SpectroMonitor III, Model 1204A detector (Laboratory Data Control, Riviera Beach, FL), a Rheodyne Model 7125 sampling valve having a 20.0 μL fixed loop (Rheodyne, Inc., Cotati, CA) and a HP Model 3380 integrator (Hewlett-Packard, Avondale, PA). A 30 cm x 3.9 mm I.D. μBondapak C_{18}, 10 micron column (Waters Associates, Milford, MA) was used.

Typical operating conditions: mobile phase flow rate 1.00 mL/min at 870 psi, detector at 280 ηm, sensitivity 0.05 A.U.F.S., temperature ambient and chart speed 0.5 cm/min.

Mobile Phase

The mobile phase composition was 70% v/v methanol, 1%v/v acetic acid in water and having an overall concentration of 5×10^{-3} M 1-pentanesulfonic acid, sodium salt. prepared by combining 700 mL of methanol, 10 mL of acetic acid, and 0.961 g of 1-pentanesulfonic acid, sodium salt, diluting to about 1L with distilled water, cooling to room temperature and adjusting to volume with water. The apparent pH of the mobile phase was 3.50. Prior to use, the mobile phase was filtered through a 47 mm diameter, 0.45μm porosity nylon-66 membrane (AMF/CUNO, Meriden, CT).

Dilution Solvent

70% v/v methanol and 1% v/v acetic acid in water.

Standard Solutions

A standard stock solution was prepared at a concentration of 0.400 mg/mL by dissolving 40.0 mg of the USP Oxymetazoline Hydrochloride reference standard in Dilution Solvent. A working standard was prepared daily at a concentration of 40.0 µg/mL by further dilution of the stock standard in Dilution Solvent.

Procedure

Bulk Drug Substances: An accurately weighed portion of drug substance was dissolved in and diluted with Dilution Solvent to a final concentration of 40 µg/mL.

Aqueous Samples: Aliquots of sample based on the label claim equivalent to 4mg of oxymetazoline HCl were diluted to 100.0 mL with Dilution Solvent.

Each of the sample preparations was passed through a 0.45µm nylon-66 membrane filter and chromatographed under the conditions described above. Duplicate sample injections bracketed by standard injections were made and quantitation achieved by comparison of the average chromatographic responses.

Fortified Commercial Samples

Each sample was fortified at the 100% level with oxymetazoline HCl reference standard and assayed by the proposed procedure. Fortification was carried out by taking a volume of sample based on the label claim equivalent to 2.00 mg of oxymetazoline HCl adding 5.00 mL of the standard stock solution and diluting to 100.0 mL with Dilution Solvent.

Synthetic Formulations

Formulation A, (Representing a 0.05%w/v commercial formulation): An aqueous solution was prepared containing in each mL, glycine (3.8 mg), benzalkonium chloride (0.2 mg), sorbitol solution USP (57 mg) and phenylmercuric acetate (0.02 mg). The pH was adjusted to 6.0 with 0.1N

sodium hydroxide. This solution was used as a diluent in preparing formulations containing 0.04%, 0.05% and 0.06% oxymetazoline HCl representing 80%, 100% and 120% respectively of the label claim.

Formulation B, (Representing a 0.025% w/v commercial formulation): An aqueous solution was prepared as above with the pH adjusted to 4.50 with 0.1N HCl. This solution was used as diluent in preparing formulations containing 0.02%, 0.025% and 0.03% oxymetazoline HCl representing 80%, 100% and 120% respectively of the label claim.

These synthetic formulations were assayed by the proposed procedure.

RESULTS AND DISCUSSION

A drug product quality survey of various adrenergic stimulants of pharmaceutical interest including oxymetazoline required the need for a rapid, rugged and accurate method for the analysis of commercial formulations. The use of reversed-phase ion-pairing HPLC was investigated and selected as a means for the isolation and quantification of oxymetazoline HCl. We have found this technique to be an attractive alternative to ion-exchange chromatography for the analysis of numerous medicinal agents representing both acidic and basic ionizable species. It is quite versatile with respect to selectivity which can be readily changed through minor modifications in mobile phase composition under ambient conditions.

Sample Analysis

Four samples of oxymetazoline HCl in bulk drug form obtained from three sources were assayed by the proposed HPLC procedure and by a non-aqueous titration (19). The comparative assay results are shown in Table 1. The two procedures provided similar assay values and all were within the USP XXI purity limits of 98.5% to 101.5% calculated on the dried basis.

TABLE 1

Analysis of Bulk Drug Substances

	Oxymetazoline HCl Found, % *	
	USP XX Titration	Proposed HPLC Procedure (n=3)
Manufacturer-A		
Sample #1	99.28%	99.2%
Sample #2	99.12%	100.0%
Manufacturer-B	99.00%	99.5%
Manufacturer-C	98.96%	98.3%

* Calculated on the dried basis

Twenty-one commercial formulations representing ten different manufacturers were assayed in triplicate by the proposed procedure. Two of the products were labeled to contain 0.025%w/v oxymetazoline HCl and the remainder were formulated at the 0.05% level. A compilation of the analytical results is presented in Table 2. The assay values ranged from 94.4% to 103.2% of the declared concentration and all products were found to be within the USP XXI limits of 90.0% to 110.0%. The coefficient of variation (CV) based on the replicate determinations ranged from 0.11% to 2.02%.

None of the chromatograms in this series of samples were found to exhibit interferences with the response for oxymetazoline HCl. Typical chromatograms representing a reference standard solution and a diluted product are shown in Fig. 2. The product in this case was preserved with 0.001% thimerosal. Common ingredients and preservatives used in formulating this type of nasal decongestant are: glycine, sorbitol, camphor, menthol, eucalyptol, benzalkonium chloride, phenylmercuric

TABLE 2

Analysis of Commercial Formulations- 0.05% Oxymetazoline HCl, n=3

		Found		
Sample	Brand	% w/v	% of Declared	C.V.,%
1	A	0.0500	100.0	0.20
2	A	0.0503	100.6	1.00
3	A	0.0499	99.8	2.02
4	A	0.0509	101.8	0.86
5	A	0.0504	100.8	0.80
6	B	0.0481	96.2	0.24
7	B	0.0484	96.8	0.43
8	B	0.0486	97.2	0.31
9	B	0.0500	100.0	1.12
10	B	0.0492	98.4	1.17
11	C	0.0484	96.8	1.38
12	C	0.0475	95.0	0.49
13	C	0.0477	95.0	0.21
14	D	0.0516	103.2	0.56
15	E	0.0499	99.8	0.70
16	F*	0.0236	94.4	2.00
17	F*	0.0242	96.8	0.24
18	G	0.0481	96.2	0.62
19	H	0.0505	101.0	0.11
20	I	0.0507	101.4	0.52
21	J	0.0504	100.8	0.30

* Formulated at the 0.025% level

acetate and thimerosal. Individual examination of these components under the described chromatographic conditions at concentrations usually present in this type of product also indicated no interfering responses. Thimerosal was found to exhibit a response at 6.1 min. However, during this study, none of the products declaring this preservative exhibited a response corresponding to that of a thimerosal reference standard. One of the products (Fig. 2-B) showed

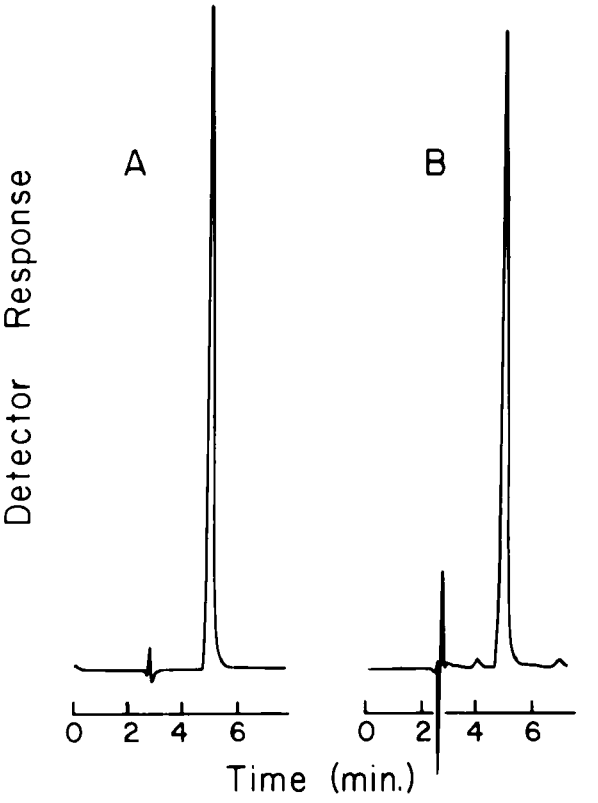

FIGURE 2.

Chromatograms obtained by the proposed procedure for oxymetazoline hydrochloride standard solution (A) and a diluted nasal decongestant formulation (B). Conditions described under Experimental.

chromatographic evidence for the presence of the primary and secondary degradation species of thimerosal. These minor responses depicted in Fig. 2-B represent thiosalicylic acid (~4 min) and 2,2' dithiosalicylic acid (~7 min), respectively. The loss of thimerosal under various conditions in aqueous media through either decomposition or adsorption onto the walls of plastic containers has been previously reported (20).

TABLE 3

Recovery Data for Oxymetazoline HCl from Fortified Commercial Formulations

Brand	Added (mg)	Recovered (mg)	% Recovered
A	2.00	2.01	100.5
B	2.00	1.98	99.0
C	2.00	1.99	99.5
D	2.00	2.00	100.0
E	2.00	1.95	97.5
F	2.00	2.00	100.0
G	2.00	1.98	99.0
H	2.00	2.00	100.0
I	2.00	1.98	99.0
J	2.00	1.98	99.0

Recovery Study

The accuracy of the procedure was evaluated by several means. One sample from each of the ten manufacturers was fortified with oxymetazoline HCl at the 100% level and assayed by the proposed procedure. The recovery values are presented in Table 3 and range from 97.5% to 100.5% with an overall average of 99.4%.

Multi-level synthetic formulations representing a 0.05%w/v (Formulation A) and a 0.025%w/v commercial product (Formulation B) were also assayed by the proposed procedure. The recovery data obtained from these simulated preparations presented in Table 4 ranged from 99.5% to 101.0% for Formulation A and 97.0% to 99.0% for Formulation B.

Precision and Linearity

In addition to the CV values reported for the replicate sample analyses, the method precision was evaluated by repeated assays of one commercial formulation over separate periods of one day and one

TABLE 4

Recovery of Oxymetazoline HCl from Synthetic Formulations

	Formulation level (%w/v)	Found (%w/v)	% Recovery
Formulation-A	0.04	0.0404	101.0
	0.05	0.0503	100.6
	0.06	0.0597	99.5
Formulation-B	0.020	0.0194	97.0
	0.025	0.0248	99.2
	0.030	0.0297	99.0

week. The within-day precision was determined by performing seven consecutive assays within a period of eight hours. The day-to-day repeatability of the method was determined by analyzing the same sample (single operator) on seven consecutive days. These measurements of precision provided CV values of 0.334% and 1.059%, respectively, as shown in Table 5.

Under the described chromatographic conditions, a linear response was demonstrated for oxymetazoline HCl over at least a ten-fold range in concentration (8-80µg/mL) with a correlation coefficient of 1.000.

Method Comparability Study

The current official method for the analysis of oxymetazoline HCl drug substance and nasal solutions containing this decongestant is an HPLC procedure employing cation-exchange chromatography (21). A typical chromatogram of a reference standard solution and sample obtained by this technique is shown in Fig. 3. Four nasal preparations each from a different source were assayed by the proposed ion-pairing procedure and by the USP XXI procedure. A comparison of the assay

Table 5

Precision of Method: Repeatability Data, (n=7)

Determination		Oxymetazoline HCl Found, % w/v	
		Within-Day	Day-to-Day
1		0.0512	0.0514
2		0.0516	0.0522
3		0.0513	0.0526
4		0.0514	0.0517
5		0.0514	0.0522
6		0.0515	0.0526
7		0.0517	0.0530
	Mean	0.0514	0.0522
	Range	0.0512-0.0517	0.0514-0.0530
	C.V. (%)	0.334	1.059

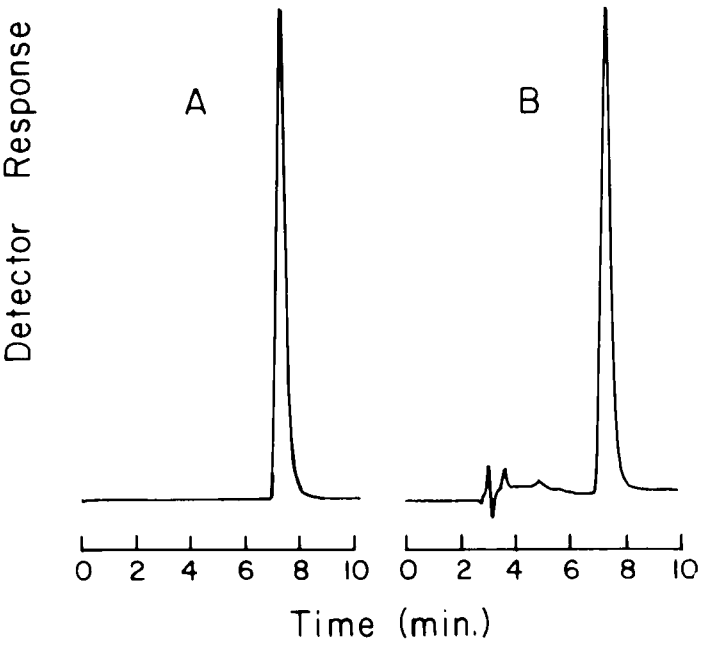

FIGURE 3.

Chromatograms obtained by the USP XXI procedure for oxymetazoline hydrochloride standard solution (A) and a diluted nasal decongestant formulation (B). Column: Partisil 10 SCX, 25 cm x 4.6 mm I.D., 10 µm. Mobile phase: water-methanol-1M sodium acetate-acetic acid (46:40:10:4), pH 4.4. Flow rate: 1.0 mL/min. Wavelength: 280 ηm. Temperature: 26° C.

TABLE 6

Comparative Results of Analysis: USP XXI and Proposed Procedure

	Oxymetazoline Found, % w/v	
	USP Procedure	Proposed Procedure
Sample-1	0.0511	0.0506
Sample-2	0.0490	0.0488
Sample-3	0.0251	0.0252
Sample-4	0.0497	0.0488

values presented in Table 6 indicates that both methods provide similar results and could be used interchangeably. The reversed-phase ion-pairing technique does offer several advantages with respect to column stability, analyte peak shape and overall versatility. The proposed method with minor changes in mobile phase composition has been successfully adapted by our laboratory for the analysis of other imidazoline derivatives in commercial formulations including xylometazoline, tetrahydrozoline and naphazoline.

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