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Ethyl Chloroformate as Derivatizing Reagent for Gas Chromatographic Determination of Norephedrine, Ephedrine and Pseudoephedrine in Pharmaceutical Preparation

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An analytical procedure has been developed for the gas chromatographic determination of norephedrine, ephedrine and pseudoephedrine using ethyl chloroformate as derivatizing reagent. Each of the compounds indicated single peak with mass spectrum corresponding to the derivative. The elution and separation was carried out from the column HP-5 (30 m × 0.32 mm i.d.) with film thickness 0.25 µm and detection was by flame ionization detector. The linear calibration curves were obtained with 47.5 to 475 µg/mL norephedrine, 50-500 µg/mL ephedrine and pseudoephedrine with detection limits of 11.2, 12.5 and 10 µg/mL, respectively. The method was applied for the determination of norephedrine, ephedrine and pseudoephedrine from pharmaceutical preparations and relative standard deviation (RSD) for the analysis of pharmaceutical preparation was obtained within 0.1 to 0.8 %. A number of additives present in the pharmaceutical preparations did not affect the determination. The % recovery of the norephedrine from pharmaceutical preparations was within 96.6-98.5 % with RSD 0.1-0.3%.

Key Words: Gas chromatography, Pseudoephedrine, Ephedrine, Norephedrine.

INTRODUCTION

Norephedrine (NEP), ephedrine (EP) and pseudoephedrine (PEP) are potential central nervous system stimulants and are listed as banned substances by International Olympic Committee, because of their stimulating properties for instance, reduced tiredness and increased alertness. These substances are also commonly used in the treatment of cold and allergy[1]. However their use has resulted into a number of side effects including hemorrhagic stroke, arrhythmias and hypertention[2,3]. EP and NEP have comparatively more cardiovascular stimulant effect than PEP. Their combination would be expected to have more adverse cardiovascular events such as hypertension or myocardial ischemia[4].

Severe adverse effects related to these products have been reported to US Food and Drug Administration and resulted in a federal ban on supplements that contain

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ephidra[5,6]. The development of simple analytical procedure involving inexpensive chemicals for the determination of NEP, EP and PEP from pharmaceutical preparations for quality control could be of interest. A number of analytical methods have been reported for the determination of NEP, EP and PEP, but the recent methods are based on capillary electrophoresis[7], gas chromatography (GC)[8-12], liquid chromatography (LC)[13-17] and LC-mass spectrometry[18,19]. The GC methods are based on without derivatization[20,21] or precolumn derivatization with N-methyl-N-trimethylsilyltrifluoroacetamide[9], N-methyl-*bis*-(heptafluorobutyramide)[12,22], heptafluorobutyric anhydride[6,9,10,23], pentafluoropropinic anhydride[24] and trifluoroacetylacetone[25]. However the uses of acetylating reagent affect the performance of GC column. Ethyl chloroformate (ECF) has been used as gas chromatographic reagent for amines, amino alcohols and acids[26]. Chloroformates in gas chromatography as general purposes derivatizing reagent and their strategies for GC analysis on a decade use as esterifying agent have been reviewed[27,28]. 2,2,2-Trichloroethyl chloroformate has been used as derivatizing reagent for NEP, EP and PEP[29] and methyl chloloformate for NEP and EP[30]. However NEP and PEP coeluted[28]. The present work examined the use of ECF as a reagent for the determination of NEP, EP and PEP by GC and confirmed the formation of derivatives by GC-mass spectrometry (MS) and determined by GC-flame ionization detection (FID).

EXPERIMENTAL

Ethyl chloroformate (ECF) (Fluka, Switzerland), phenylpropanolamine hydrochloride (norephedrine hydrochloride) (Sandoz Pak. Ltd., Karachi), ephedrine hydrochloride (1-methylaminoethylbenzylalcohol hydrochloride) (E. Merck, Germany), pseudoephedrine hydrochloride (Novartis Ltd. Pak), methanol and chloroform (E. Merck, Germany) and sodium chloride (Fluka, Switzerland) were used. The buffer solutions within pH 1-12 at unit interval were prepared from the following: Hydrochloric acid (0.1 M) and potassium chloride (0.1 M), (pH 1-2), acetic acid (0.1 M) and sodium acetate (0.1 M) (pH 3-6), ammonium acetate (0.1 M) (pH 7), sodium bicarbonate (0.1 M) and sodium carbonate (0.1 M) (pH 8-9), ammonium chloride (0.1 M) and ammonia (0.1 M) (pH 10-11) and sodium chloride (0.1 M) and sodium hydroxide (0.1 M) (pH 12). Pharmaceutical preparation tavegyl-D (Sandoz Pak. Ltd. Karachi), sinutab (Parker Davis and Co. Pak. Ltd. Karachi), ephedrine tablets (Karachi Chemical Ltd. Karachi), actifid-P (Glaxo-Smith-Kline (Pak.) Karachi), Tandegyl (Novartis (Pak.) Karachi), telfast (Aventis Pharmaceutical Pak. Karachi), arinac (Abbot laboratories Ltd, (Pak.) Karachi) and panadol CF (Pharmatec Pak. (Pvt) Ltd) were purchased from local market (Hyderabad).

Spectrophotometric studies were carried out using a double beam Hitachi 220 (Hitachi (Pvt.) Ltd. Tokyo, Japan) spectrophotometer with dual quartz cuvettes with 1 cm path length. pH measurements were made with Orion 420 A pH meter (Orion Research Inc. Boston, USA) with glass electrode and internal reference electrode.

The gas chromatographic studies were carried out on Agilent model 6890 Network GC system gas chromatograph (Agilent Technologies Inc. USA) coupled with flame ionization detection (FID), split/splitless injector operated in split mode, hydrogen generator Parker Balson Model H2-90, Analytical gas system (Parker Hannifin Haverhill, M.A. USA) and pure nitrogen (British Oxygen Company (BOC) Karachi). The gas chromatograph was controlled by the computer with Chemstation software (Agilent Technologies), HP 1300 Laser jet was used throughout the study. Capillary GC column HP-5 (5 % phenyl methyl siloxane) (30 m × 0.32 mm i.d.) with film thickness 0.25 μm (J & W Scientific GC column, USA) was used throughout the study.

GC-MS studies were carried out using GC mass selective detector (MSD) composed of Agilent model 6890 GC interfaced with Agilent 5957 mass selective detector/ qudrapole mass spectrometer. The system used electron impact ionization at 70 eV and was capable of performing full mass scan or selective ion monitoring (SIM) modes. The system was coupled with Agilent 7683 auto injector with split/splitless injector, operated in split-mode, Parker Balson nitrogen generator, Analytical gas system Model UHPN2-1100 (Parker Hanniton Corp. Haver hill, M A, USA). GC-MS was controlled by computer with Chemstation software (Agilent Technologies Inc. USA).

GC Operating conditions

GC-FID: The solution (1 μL) was used and eluted from column HP-5 (30 m × 0.32 mm i.d.) with film thickness 0.25 μm at column temperature 70 °C for 10 min, followed by heating rate 15 °C/min upto 130 °C with total run time 34 min. Injection port and detector temperatures were maintained at 270 and 300 °C. Nitrogen flow rate was 2 mL/min with split ratio 10:1. The detection was performed by FID. Nitrogen was used as make up gas with flow rate 45 mL/min. The hydrogen and air flow rates for FID were maintained at 45 and 450 mL/min, respectively.

GC-MS: The solution (1 μL) was used and eluted from column HP-5 (30 m × 0.32 mm i.d.) with film thickness 0.25 μm at initial column temperature 90 °C for 1 min, followed by heating rate 10 °C/min upto 140 ºC with total run time 20 min. The nitrogen flow rate was 3.5 mL/min with split ratio 100:1. The temperatures were adjusted as follows: Inlet 250 °C, auxiliary 260 °C, MS source 230 °C and MS at 150 °C. The elution of NEP, EP and PEP as ethyl chloroformate derivatives was carried out separately.

Analysis of NEP, EP and PEP from pharmaceutical preparation: Ten tablets each tavegyl-D and Sinutab for the analysis of NEP were well ground. Tavegyl-D 0.512 g and sinutab 0.475 g corresponding to one tablet each were dissolved in hydrochloric acid (0.01 M) separately. The solution was filtered and volume was adjusted to 10 mL.

Five ephedrine tablets were powdered and amount corresponding to one tablet (0.1612 g) was weighed and dissolved in hydrochloric acid (0.01 M). The solution was filtered and volume was adjusted to 10 mL. Five tablets each of actifid-P,

tandegyl, telfast, arinac and panadol CF for PEP were well ground and amount equivalent to one tablet actifid-P (0.601 g), telfast (0.807 g), tandegyl (0.524 g), arinac (0.727 g) and panadol CF (0.671 g) were weighed and dissolved in hydrochloric acid (0.01 M). The solution was filtered and volume was adjusted to 100 mL. The solution (0.1 mL) from travegyl-D and sinutab; 0.2 mL from ephedrine, tandegyl, telfast, arinac, panadol CF or 0.4 mL actifid P tablets was added carbonate buffer (pH 9) (1 mL) and ethyl chloroformate (0.5 mL). The contents were mixed well for 2 min and reaction mixture was allowed at room temperature (30 ºC) for 15 min. Chloroform (0.5 mL) was then added and the mixture was shaken vigorously. The layers were allowed to separate and a portion of the extract obtained (1 mL) was transferred to screw capped vial. The solution (1 µL) was eluted as 2.3.1 for GC-FlD determination. The amount of drug from pharmaceutical preparation was calculated using external calibration curve-prepared from standard solution containing 47.5-475 µg/mL NEP, 50-500 µg/mL EP or PEP.

% Recovery of NEP from pharmaceutical preparations: Well ground samples of tavegyl-D (0.327 g) and sinutab (0.913 g) were weighed and dissolved in hydrochloric acid (0.01 M). The solution was filtered and volume was adjusted to 25 mL. The solution (0.2 mL) from each of the solution was processed as described above. Three solutions (0.2 mL) each was added 190, 380 and 570 µg NEP in sequence to the solution of both tavegyl-D and sinutab. The analysis was carried out using analytical procedure as described above. The quantitation was carried from the enhancement of the response from calibration curve and by graphical method.

RESULTS AND DISCUSSION

Optimization of reaction conditions and separation: The compounds NEP, EP and PEP containing primary and secondary amino groups reacted with ethyl chloroformate to form carbamates (Fig. 1). The formed derivatives were sufficiently volatile to elute from capillary GC column HP-5. The effects of pH, concentration of derivatizing reagent, solvent and reaction time on derivatization were examined. Each time constant volume (1 µL) was injected and average peak height/peak area (n = 4) was noted. The condition, which gave maximum response, was considered as optimum. The effect of pH was examined within 1-12 at unit interval and maximum response was observed in alkaline medium at pH 9, as have been reported for amino containing compounds[23,24]. The carbonate buffer used covered the pH 9 satisfactory (Fig. 2). The addition of pure derivatizing reagent ethyl chloroformate was varied from 0.1 mL to 0.6 mL at an interval of 0.1 mL. The addition of ethyl chloroformate was not observed critical as long excess was available and a similar response was observed with addition of 0.2 mL and above. For quantitative response addition of 0.5 mL was selected. The reactions were examined in methanol, acetonitrile, aqueous solution and aqueous solution containing pyridine base. The aqueous medium in the presence of carbonate buffer proved a better choice. The reactions were carried out at room temperature 30 °C with reaction time within 5 to 20 min at an interval

of 5 min and reaction time of 15 min was selected (Fig. 3). A quantitative reaction was observed with reproducible response for each of the compound with related standard deviation (RSD) within 2 %.

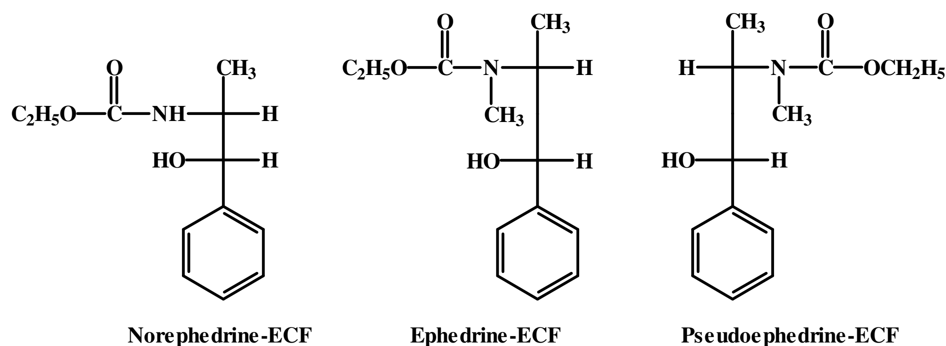

Fig. 1. Structural diagrams of ethyl chloroformate (ECF) derivative of (1) NEP, (2) EP and (3) PEP

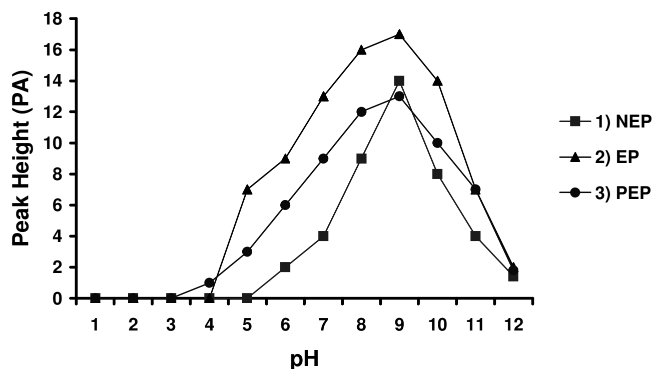

Fig. 2. Effect of pH on derivatization of NEP, EP and PEP with ethyl chloroformate

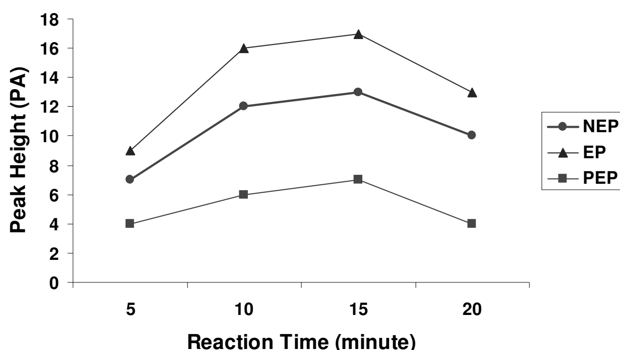

Fig. 3. Effect of reaction time on derivatization of NEP, EP and PEP with ethyl chloroformate

The elution of NEP, EP and PEP as derivatives of ethyl chloroformate was confirmed by recording GC-MS for each derivative separately (conditions as GS-MS). Each of the derivative indicated a single peak and NEP indicated molecular ion peak (M^+) at m/z 223 (Fig. 4) and PEP, EP have same molecular mass and indicated signals at m/z 219 due to loss of H_2O from molecular ion peaks (Figs. 5 and 6). However when concentration of PEP derivative was increased it indicated (M^+) at m/z 237. If may be suggested that derivatization with ethyl chloroformate involved only primary and secondary amino group, but did not interact with alcoholic OH group. The NEP derivative indicated main peaks at m/z at 107 and 116 corresponding to $[C_6H_5CHOH]^+$ and $[C_2H_5OCONHCH(CH_3)]^+$ fragments, respectively. EP and PEP derivatives indicated base peaks at m/z 130 due to the loss of C_6H_5CHOH fragment from molecular ion peak.

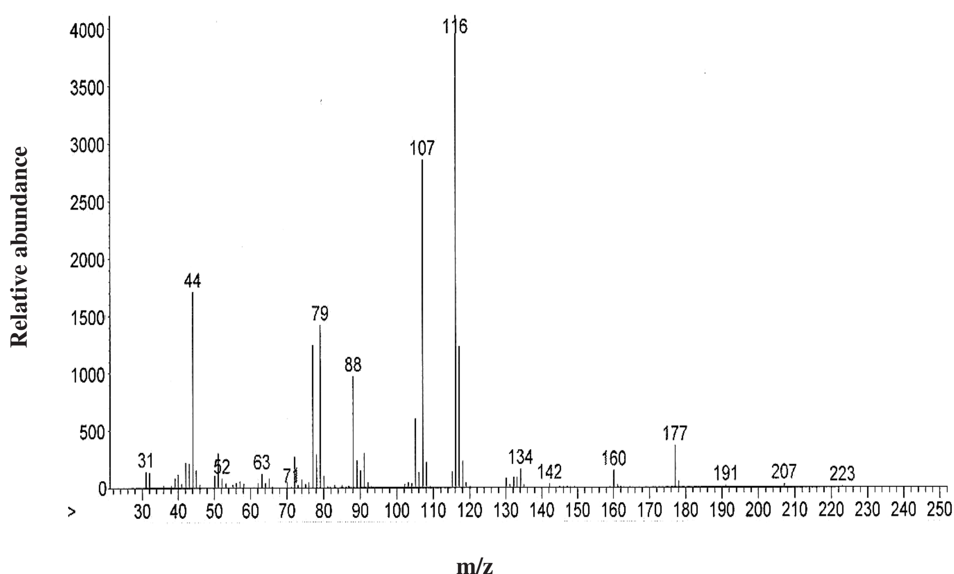

Fig. 4. GC-MS of NEP as derivative of ethyl chloroformate

The separation of NEP, EP and PEP from GC column HP-5 was then examined. The separation of NEP from EP and PEP was easily obtained, but some overlapping between peaks of EP and PEP was obtained. However a separation was obtained at GC-FID operating conditions with resolution factor (Rs) between EP and PEP observed 0.7. A variation in oven temperature or nitrogen flow rate did not improve the separation of EP and PEP derivatives. The formation of ethyl chloroformate derivatives of NEP, EP and PEP, confirmed by GC-MS and separation of NE, EP and PEP by GC-FID could be used for qualitative and quantitative identification of the drugs from pharmaceutical preparations.

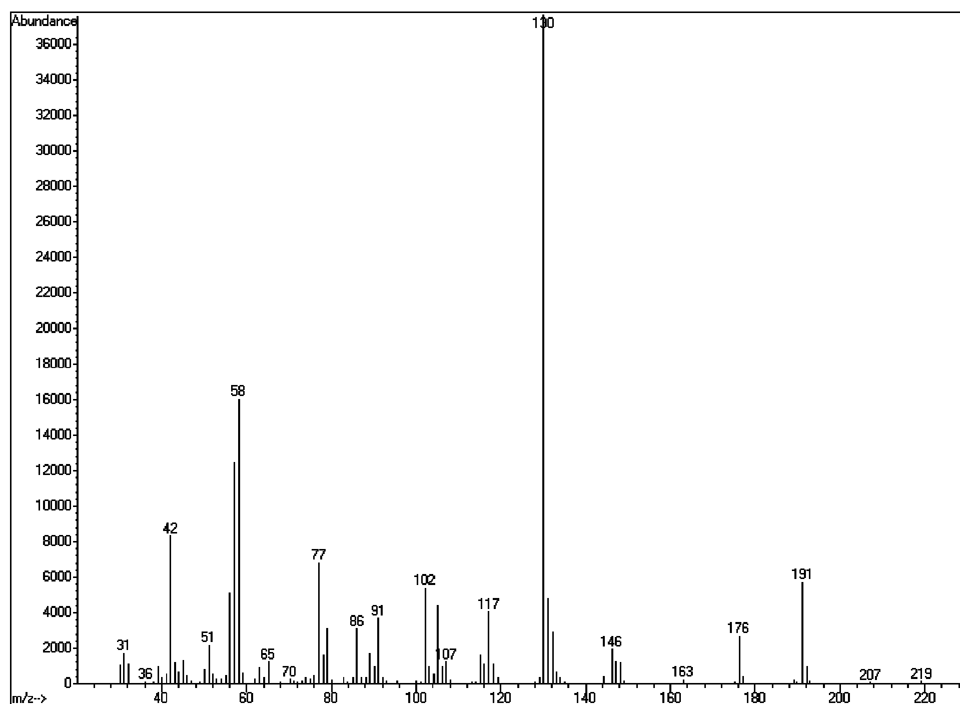

Fig. 5. GC-MS of PEP as a derivative of ethyl chloroformate

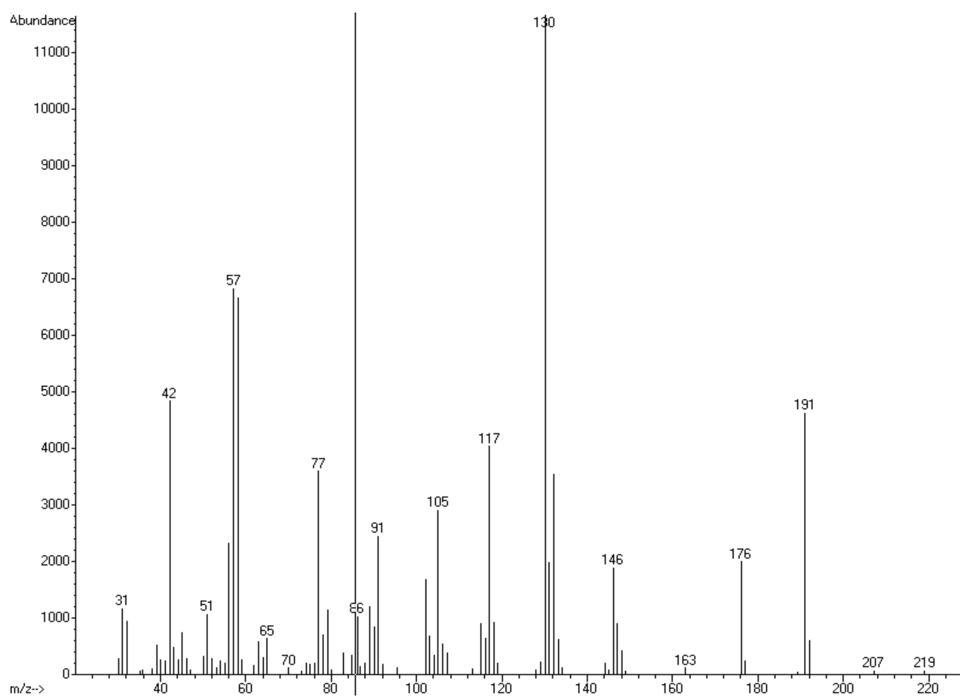

Fig. 6. GC-MS of EP as a derivative of ethyl chloroformate

Quantitation and validation: Linear calibration curves were obtained by plotting average peak height/peak area (n = 4) *versus* concentration of NEP, EP and PEP with 47.5-500 µg/mL (Table-1). The limit of detection (LOD) measured as signal to noise ratio 3:1 corresponded to 11.2 ng, 12.5 ng and 10 ng/ injection (1 µL) and 1.12, 1.25 and 1.0 ng reaching to detector (split ratio 10:1) for NEP, EP and PEP, respectively. The limit of quantitation (LOQ) measured as three time LOD was within 30-37.5 µg/mL.

TABLE-1
GC PARAMETER FOR NEP, EP AND PEP AS ETHYL CHLOROFORMATE DERIVATIVES

Compd.	Calibration range (µg/mL)	Coefficient of determination	Linear regression equations	Limit of detection (µg/mL)	Limit of quantitation (µg/mL)
NEP	47.5–475	0.9986	Y= 0.1153 x + 1.15	11.2	33.6
EP	50–500	0.9982	Y= 0.1212 x + 0.08	12.5	37.5
PEP	50–500	0.9830	Y= 0.1345 x + 1.39	10.0	30.0

The precision for repeatability of the determination of NEP (190 µg/mL), EP and PEP (200 µg/mL) in terms of average peak height/peak area and retention time (n = 6) was examined and relative standard deviation (RSD) was observed within 1.2-1.8 and 1.1-1.4 %, respectively. The effects of additives present in the pharmaceutical preparation were examined for possible interfering effect on the determination of NEP, EP and PEP. Magnesium stearate, gum acacia, methylparaben, lactose, starch, glucose and talcum when added twice the concentration of NEP, EP or PEP, did not affect the determination with relative error within ± 3 % (n = 4). Clemastine hydrogen fumerate and phenyltoloxamine citrate are commonly present in pharmaceutical preparation together with NEP. Their effects on the determination of NEP were examined. NEP is selectively extracted from alkaline medium in chloroform after derivatization with ethyl chloroformate and eluted from GC column but clemastine hydrogen fumerate and phenyltoloxamine citrate did not elute form GC column and did not interfere the GC determination of NEP. Four test solutions of different concentrations of NEP were analyzed to cover the calibration range and relative % error was obtained within ± 2.5 %.

Sample analysis: NEP, EP and PEP are separately present in pharmaceutical preparations and their determinations were examined. NEP was examined in tavegyl-D and sinutab tablets. EP contents were analyzed from ephedrine tablets and PEP was assessed in actifed-P, telfast, tandegyl, arinac and panadol CF tablets. The results of analysis are summarized in Table-2 and indicate a close correlation with the labeled values. The results indicate RSD within 0.78 %. % Recovery of NEP was examined from tablets tavegyl-D and sinutab by standard addition and was observed in the range of 97.8-98.2 and 96.6- 98.6 % with RSD 0.1-0.3 and 0.2-0.2 %, respectively.

TABLE-2
RESULT ANALYSIS OF NEP, PEP AND EP FROM PHARMACEUTICAL PREPARATIONS AS ETHYL CHLOROFORMATE DERIVATIVES

Name of compound	Name of drug	Amount labeled mg/tablet	Amount found mg/tablet (RSD %) (n = 4)
Norephedrine hydrochloride	Tavegyl-D	75	74.3 (0.1)
	Sinutab	25	23.9 (0.3)
	Telfast-D	120	117.0 (0.2)
Pseudoephedrine hydrochloride	Arinac	60	56.6 (0.3)
	Actifed - P	36	34.3 (0.5)
	Panadol CF	60	58.0 (0.2)
	Tandegyl-D	90	85.0 (0.2)
Ephedrine hydrochloride	Ephedrine	30	28.5 (0.8)

Conclusion

The work reports simple GC procedure for the biological active compounds NEP, EP and PEP using ethyl chloroformate as derivatizing reagent. The detection limits were obtained with 10-12.5 ng/injection. The method has been applied for the analysis of NEP, EP and PEP from pharmaceutical preparations with RSD within 0.8 % and results correlated with expected values.

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