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Liquid chromatographic method for the determination of nicotine in pharmaceutical formulations

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Abstract

A simple high-performance liquid chromatography method was developed and validated for the analysis of nicotine in various pharmaceutical formulations. This method required a simple liquid–liquid extraction procedure prior to liquid chromatography analysis. The chromatographic separation was achieved on a reversed-phase C_{18} column with ultraviolet detection at 260 nm. This isocratic system was operated at ambient temperature and required 10 min of chromatographic time. The mobile phase consisted of methanol–citrate phosphate buffer (15:85, v/v) at a flow-rate of 0.7 ml/min. Standard curves were linear over the concentration range 1.0–51 μg/ml. Within-day and day-to-day relative standard deviations ranged from 1.3 to 4.4% and from 2.4 to 4.2%, respectively.

Keywords: Nicotine

1. Introduction

Nicotine is a cholinomimetic drug obtained from the plant *Nicotina tabacum*. It is a weak base and its molecular structure is shown in Fig. 1. It is available as a colorless to pale yellow oily liquid with an unpleasant tobacco-like odor and burning taste. The pK_a values of this tertiary base are 6.16 (pK_1) and 10.96 (pK_2) at 15°C [1]. Nicotine is the only FDA approved pharmacologic agent for use in smoking-cessation therapy. Currently two forms of nicotine formulations are available: nicotine chewing gum and nicotine transdermal system. Various liquid chromatography (LC) [2–6] and gas chromatography (GC) [7,8] methods have been reported for the analysis of nicotine and its metabolites in blood and urine. All the LC methods reported for the analysis of nicotine used expensive mobile phase systems and complex extraction procedures. No method is currently available to quantitate nicotine in both these pharmaceutical formulations. However, the first sup-

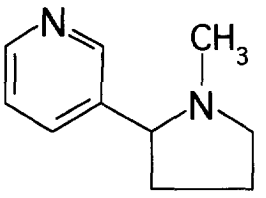

Fig. 1. Structure of nicotine.

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plement to the United States Pharmacopeia (U.S.P.) XXIII has included a LC method for the analysis of nicotine in nicotine gum [9]. This ion-pairing chromatographic method also uses an expensive mobile phase system and can not be used for the analysis of nicotine in transdermal systems without modification [10]. Therefore, the object of this investigation was to develop a simple LC method which can be used for the analysis of nicotine in aqueous solution and in all the available pharmaceutical formulations.

2. Experimental

2.1. Materials

Nicotine and lidocaine hydrochloride (Sigma, St. Louis, MO, USA), nicotine gum (Nicorette, Lakeside Pharmaceuticals, Cincinnati, OH, USA), nicotine transdermal systems (Nicoderm, Marrion Merrel Dow, Kansas City, MO, USA), anhydrous citric acid, *n*-hexane, methanol, perchloric acid, sodium phosphate (monobasic), HPLC grade water (Fisher Chemical, Fairlawn, NJ, USA) were used as received.

2.2. Chromatography

The high-performance liquid chromatography (HPLC) system comprised a pump (Model LC-600) programmed by a system controller (Model SCL-6B), an UV-Vis spectrophotometric detector (Model SPD-6AV) and a recorder (Model CR-501), all from Shimadzu (Tokyo, Japan). The separation was carried out on a 150×4.6 mm I.D. S5 PC18 column (Phase Separations, Norwalk, CT, USA). The mobile phase was methanol–citrate phosphate buffer (15:85, v/v, apparent pH 2.4 adjusted with addition of perchloric acid) and the flow-rate was 0.7 ml/min. The column effluent was monitored at 260 nm.

2.3. Solutions

Solution A: Monobasic sodium phosphate (14.19 g) was dissolved in water (HPLC grade) and the volume made up to 500 ml. Solution B: Anhydrous citric acid (19.21 g) was dissolved in water (HPLC grade) and the volume made up to 1000 ml. Citrate phosphate buffer was prepared by mixing solution A (354 ml) to solution B (646 ml) with constant stirring. Nicotine standard solutions (1.0 to 50.8 μg/ml) were prepared in mobile phase. Internal standard solutions (Lidocaine hydrochloride solution 1.28 mg/ml) were prepared in methanol.

2.3.1. Mobile phase

Methanol (150 ml) was mixed with 850 ml of citrate phosphate buffer. The solution was filtered through a 0.45-μm MAGNA Nylon filter (MSI, Westboro, MA, USA).

2.3.2. Sample preparation for LC

Internal standard solution (60 μl) was added to a borosilicate culture tube and evaporated to dryness at 40°C in an oven. Standard solution (500 μl) was spiked to the test tube and vortexed for 15 s. An aliquot (20 μl) was analyzed by LC.

2.3.3. Calculation

The ratios of the peak heights of nicotine to that of the internal standard were calculated. The unknown nicotine concentration was determined from the regression equation relating the peak-height ratio of the standards to their concentrations.

2.4. Analysis of nicotine formulations

2.4.1. Nicotine gum

Nicotine gum (2 mg) was cut into 16 small pieces and placed in an separatory funnel. The gum pieces were dissolved in 20 ml hexane (HPLC grade) for 24 h. Nicotine was extracted from *n*-hexane 5 times with 20 ml of mobile phase each time. The extracts were collected in 100-ml, 50-ml, 50-ml, 50-ml and 50-ml volumetric flasks, respectively. The volumes were adjusted with mobile phase. Approximately 1 ml of each of the above mixture was filtered through a 0.45-μm Nylon filter (MSI, Westboro, MA, USA) attached to a plastic syringe (Becton Dickinson, Rutherford, NJ, USA). Nicotine content in the filtrate was determined.

2.4.2. Nicotine transdermal system

Nicotine transdermal system (36 mg) without the protecting liners was placed in an separatory funnel and was immersed in 20 ml hexane (HPLC grade)

for 24 h. Nicotine was extracted 6 times with 20 ml of mobile phase each time. The extracts were collected in 100-ml, 50-ml, 50-ml, 50-ml, 50-ml and 25-ml volumetric flasks respectively. The volumes were adjusted with mobile phase. The first extraction was only diluted 10 times with the mobile phase. Approximately 1 ml of each of the above mixture was filtered through a 0.45-μm Nylon filter (MSI, Westboro, MA, USA) attached to a plastic syringe (Becton Dickinson, Rutherford, NJ, USA). Nicotine content in these solutions was determined by the HPLC.

3. Results and discussion

3.1. Assay characteristics

Fig. 2a shows a representative chromatogram of nicotine and the internal standard in mobile phase. No interfering peaks were observed in the chromatogram. Fig. 2b and c represent chromatograms of nicotine and the internal standard obtained after injection of samples prepared from Nicorette gum and Nicoderm transdermal system respectively. None of these chromatograms show any interfering peaks. The reproducibility of the retention time of nicotine (4.4 min) and lidocaine hydrochloride (6.8 min) was determined from 30 consecutive injections during an analysis of a series of nicotine samples. The relative standard deviation (R.S.D.%) was found to be 0.87 and 0.55% for nicotine and lidocaine hydrochloride, respectively.

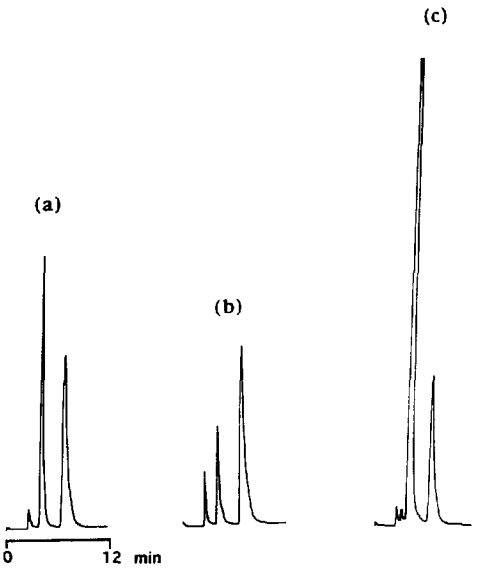

Fig. 2. Representative chromatograms obtained following injection of: (a) nicotine (5.8 μg/ml) and lidocaine hydrochloride (156 μg/ml) in mobile phase; (b) sample from Nicorette gum plus the internal standard; (c) sample from Nicoderm transdermal system plus the internal standard.

3.2. Linearity

The standard curves were linear over the concentration range of 1.0–50.8 μg/ml. The equation of the standard curve relating the peak-height ratio (P) to the nicotine concentration (C in μg/ml) in this range was: $P=0.257C-0.021$, $r^2>0.999$.

3.3. Precision

Within-day precision was determined by analysis of four different standard curves on the same day and all analyses were carried out using the same column. Day-to-day precision was determined by the analysis of the same solutions on seven different days during a period of 40 days. During this time period, the stock solution was refrigerated (4°C) and solutions for the standard curves were prepared fresh each day from the stock solution. The variability in the peak-height ratio at each concentration was used to determine the precision of the assay procedure (Table 1). Within-day and day-to-day R.S.D. values ranged from 1.3 to 4.4% and from 2.4 to 4.2%, respectively.

3.4. Accuracy

Two quality control samples (10.2 and 24.4 μg/ml) and the standard solutions were refrigerated for 40 days. These samples were analyzed several times ($n=6$) during this period and the accuracy of the assay [(measured concentration/actual concentration)·100%] was 97.8±1.1 and 95.8±2.3%, respectively. The R.S.D. was less than 2.4%.

Table 1
Within-day and day-to-day analytical precision

Concentration (μg/ml)		R.S.D. (%)		
		Within-day[a]		Day-to-day[b]
0.00		–		–
1.02		4.4		4.2
5.08		2.8		3.1
16.3		2.2		3.5
30.4		1.6		4.0
40.6		1.3		3.3
50.8		1.8		2.4
Slope	0.261±0.0028	1.1	0.257±0.0063	2.4

[a]Analyzed on the same day.
[b]Analyzed on seven different days within a period of 40 days.

3.5. Sensitivity

The sensitivity criteria were determined from six different standard curves, using the lowest limit of reliable assay measurement criteria as described by Oppenheimer et al. [11]. The critical level is the assay response above which an observed response is reliably recognized as detectable. This was 0.06±0.01 μg/ml (mean±S.D., n=6). The detection level is the actual net response which may a priori be expected to lead to detection. This was 0.12±0.03 μg/ml. The determination level, the concentration at which the measurement precision will be satisfactory for quantitative determination, was 0.31±0.07 μg/ml for a level of precision of 10% R.S.D.

3.6. Analysis of marketed formulations

Since nicotine was available either as a gum or as a transdermal system, extraction of nicotine from these formulations was essential prior to LC analysis of nicotine. Preliminary extraction studies indicated that extraction was more complete when the formulations were dissolved in a nonpolar solvent. Hexane was found to be an ideal solvent for both these formulations. Formulations were immersed in 20 ml of n-hexane and extracted with 20 ml of mobile phase. The extraction efficiency of each extract in various formulations is shown in Table 2. In the concentration range studied, a single extraction with 20 ml of mobile phase appeared sufficient for the extraction of more than 99% of nicotine from solution. However, for nicotine gum formulation, a single extraction with 20 ml of mobile phase extracted more than 91% and for transdermal systems more than 93% of nicotine from these formulations. This study further revealed that three extractions were more than enough for the extraction of most of the nicotine present in the formulation. According to U.S.P., the gum formulation should contain not less than 95% (w/w) and not more than 110% (w/w) of the labeled amount of nicotine [9]. The nicotine transdermal system must contains not less than 90% (w/w) and not more than 110% (w/w) of the labeled amount of nicotine [10]. Both these formulations were found to satisfy the U.S.P. criteria of potency.

4. Conclusions

A simple, sensitive and reproducible method was developed for the analysis of nicotine in solution and in pharmaceutical formulations. The method required a simple liquid–liquid (n-hexane–mobile phase)

Table 2
Relative extraction efficiency of nicotine with 20 ml of mobile phase

Formulation	Strength	First extract (% extracted)	Second extract (% extracted)
Solution	0.17 mg/ml	99.3±1.0[a]	2.93±0.03[a]
	0.22 mg/ml	99.7±0.6[a]	3.25±0.09[a]
	0.19 mg/ml	99.0±2.4[a]	2.64±0.01[a]
Gum	2 mg/Gum	91.6±2.3[a]	5.42±1.17[a]
Transdermal system (TDS)	36 mg/TDS	94.3±3.0[a]	7.11±1.08[a]

[a]Mean±S.D.; n=3.

extraction procedure prior to the LC analysis. This method also used less expensive mobile phase system as compared to the method outlined in the U.S.P. This method was successfully used to determine the nicotine content in both gum and transdermal systems.

References

[1] S.C. Harvey (1990) Cholinomimetic Drugs, in A.R. Gennaro (Editor), Remington's Pharmaceutical Sciences, Eighteenth Edition, Mack Publishing, Easton, PA, pp. 891.
[2] A. Sioufi, C. Parisot, N. Sandrenan and J. P. Dubois, Methods Find. Exp. Clin. Pharmacol., 11 (1989) 179–185.
[3] N.T. Lequang Thuan, M.L. Migueres, D. Roche, G. Roussel, G. Mahuzier, J. Chretien and O.G. Ekindjian, Clin. Chem., 35 (1989) 1456–1459.
[4] R. Pacifici, S. Pichini, I. Altieri, M. Rosa, A. Bacosi and A. Caronna, J. Chromatogr., 612 (1993) 209–213.
[5] P.P. Rop, F. Grimaldi, C. Oddoze and A. Viala, J. Chromatogr., 612 (1993) 302–309.
[6] P. Zuccaro, I. Altieri, M. Rosa, A.R. Passa, S. Pichini, G. Ricciarello and R. Pacifici, J. Chromatogr., 621 (1993) 257–261.
[7] P.H. Degen and W. Schneider, J. Chromatogr., 563 (1991) 193–198.
[8] M. Hariharan and T. VanNoord, Clin. Chem., 37 (1991) 1276–1280.
[9] First Supplement to The United States Pharmacopeia, XXIII revision., pp. 2485–2486. The United States Pharmacopeial Convention, Rockville, MD (1995).
[10] Pharmacopeial Forum, Vol. 21, pp. 585–589. The United States Pharmacopeial Convention, Rockville, MD (1995).
[11] L. Oppenheimer, T.P. Capizzi, R.M. Weppelman and M. Mehta, Anal. Chem., 55 (1983) 638–643.