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On-line determination of the optical purity of nicotine

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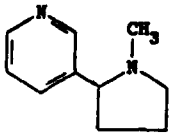
ABSTRACT

Nicotine is an alkaloid of medical importance. Precise quantitation of the enantiomeric purity of preparations of nicotine has been difficult. In the present work, a high-performance poly(styrene–divinyl benzene) reversed-phase column was used to separate the enantiomers of nicotine from other components. On-line polarimetric detection with a commercial laser polarimeter permitted sensitive, reproducible quantitation of the relative enantiomeric purity of nicotine. Detection limits of about 12 μg were established, with the range of linearity extending to about 200 μg. It was possible to assign the relative purity of mixtures of nicotine to about $\pm 0.5\%$. The precision of the on-line polarimeter was comparable to that of a static polarimeter, but the sample requirement was approximately 1000 times less. Optically inactive components were separated, making on-line polarimetry intrinsically more accurate than static polarimetry, and readily adaptable to the analysis of complex mixtures.

INTRODUCTION

Nicotine is a naturally occurring alkaloid existing as a tertiary amine with one chiral carbon located at the 2′ position of the N-methylpyrrolidine ring.



There are two optically active isomers of nicotine, the naturally-occurring (*S*)-(−)-form [1], and the (*R*)-(+)-form [2]. Although 50 species of plants contain nicotine, *Nicotiniana tabacum* and *N. rustica* [3] are the two predominant species containing nicotine. The salt form of (*S*)-nicotine has been used as a natural insecticide [4], while

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the free base has been used in medicine as an antihelminthic agent [5] and as an experimental drug in the treatment of Parkinson's [6] and Alzheimer's diseases [7]. Both isomers are toxic, exhibiting biological activity in the peripheral and central nervous systems [8]. Toxicity tests have long been performed on both isomers [9]. The (*S*)-form generally exceeds the (*R*)-form in both toxicity and biological activity. However, synergism has been noted for mixtures of (*S*)- and (*R*)-nicotine in studies on rats and guinea pigs [8,10]. There has been sustained interest in (*R*)-nicotine because its toxicity is lower than that of the (*S*)-isomer, while the biological activity is comparable.

The optical purity of (*R*)- of (*S*)-nicotine employed in biomedical studies has been difficult to quantitate. Neither repetitive diastereomeric salt purification techniques [11] nor enantiomerically selective microbial transformation of racemic mixtures [12] are readily capable of providing extremely pure materials. Precise quantitative characterization is similarly difficult. Although conventional polarimetry is suitable for purified samples, these polarimeters require relatively large samples, and are not suitable for analysis of complex mixtures such as serum. The enantiomers of a variety of alkaloids closely related to nicotine have been successfully separated by chiral chromatography, but those of nicotine itself have proved to be difficult to separate, the separation requiring 4 h and a microbore column [13–15].

Numerous chromatographic techniques and detectors have been advanced as means to differentiate and quantitate enantiomeric purity. Among the detectors that have been used in determinations of enantiomeric purity are the Perkin-Elmer 241 LC polarimeter [16–18], and laser polarimeters described by Yeung and co-workers [19,20] and Lloyd *et al.* [21]. The latter has been developed by Applied Chromatography Systems into a commercial instrument known as the ChiraMonitor. The performance characteristics of this on-line polarimeter have been described [21], as have a number of applications [22]. The present work compares static and on-line polarimetry in the analysis of mixtures of nicotine.

EXPERIMENTAL

Reagents

The (*S*)-(−)-isomer of nicotine was from Eastman Kodak (Rochester, NY, U.S.A.). The (*R*)-(+)-isomer was prepared by T.D.C. Research (Blacksburg, VA, U.S.A.) using an established method [11], substituting di-*p*-toluyl tartaric acid for tartaric acid in diastereomer formation. Triethylamine, phosphoric acid, acetonitrile, water, and 2-propanol were high-performance liquid chromatographic grade.

Instrumentation

The mobile phase was mixed and delivered by means of a Model 50 programmer and two Model 364 pumps (Knauer, Berlin, F.R.G.), a Model TCMA 0120113T Visco-Jet Mixer (Lee, Westbrook, CT, U.S.A.) and a Model LP-21 pulse dampener (SSI, State College, PA, U.S.A.). The sample was introduced with a Model 231 autoinjector (Gilson, Middleton, WI, U.S.A.) equipped with a 20-μl fixed-volume sample loop (Rheodyne, Cotati, CA, U.S.A.) onto a 25 × 0.46 cm I.D. 100 Å PLRP-S poly (styrene–divinyl benzene) reversed-phase column (Polymer Labs. Church Stretton, U.K.). The Model 750/14 ChiraMonitor and a Model 87.00 UV detector

(Knauer) were used in series as detectors. Data acquisition and transformation were accomplished by the Roseate data system (Drew Scientific, London, U.K.). The aqueous component of the mobile phase was prepared from 990 ml of 0.2% phosphoric acid, titrated to pH 7.2 with triethylamine and 10 ml 2-propanol. The organic component of the mobile phase was 990 ml acetonitrile and 10 ml 2-propanol. The aqueous and organic components were mixed by the gradient mixer in the proportion 78:22. The flow-rate was 1.0 ml/min. The mobile phase was degassed and left under a positive pressure of helium. The static polarimeter was a SEPA-200 (Horiba, Irvine, CA, U.S.A.) equipped with a sodium lamp and a 10 mm pathlength cell with a volume of 2.5 ml.

Methods

Solutions of *S*-(−)- and *R*-(+)-nicotine were prepared at a concentration of about 10 mg/ml in the mobile phase for on-line polarimetry, and at about 100 mg/ml in ethanol for static polarimetry. Duplicate 20-μl injections of enantiomeric excesses of 99.1, 96.5, 93.0, 86.1 and 73.5% of *S*-(−)-nicotine were used for on-line polarimetric spiking experiments. For static polarimetry, duplicate measurements were made of enantiomeric excesses of 100, 95.2, 85.8, 81.8, 72.4 and 64.4% of *S*-(−)-nicotine.

Calculations

Error analysis was performed by examining polarimetric response as a function of optical purity. The envelope of uncertainty around a regression line was determined by calculation of the standard error, s_Y, estimated from the expression $s_Y = \{s^2_{Y-X}[1/n + (x_i - \bar{x})^2/\Sigma x^2]\}^{1/2}$, where s^2_{Y-X} is the unexplained mean square error about the regression line, n is the number of observations, $\bar{x}$ is the mean value of enantiomeric excess, $\Sigma x^2 = \Sigma x_i^2 - (\Sigma x_i)^2$, and x_i is a particular value of enantiomeric excess [23]. An upper and lower limit for s_Y are obtained when $x_i = 100$ and $x_i = \bar{x}$, respectively. The percent standard error is the standard error expressed as a percentage of the value at the x_i of interest.

RESULTS

Fig. 1 compares the UV and ChiraMonitor traces that are obtained from (*R*)- and (*S*)-nicotine. It can be seen that both contain a small amount of an optically inactive component eluting at about 8.5 min, while the principal component elutes at about 7 min. Additional UV-positive trace components can be seen to elute between 3 and 5 min. The minor components were estimated to comprise about 5% of each sample by UV peak height. These appear to be optically inactive. It is possible to detect a component unresolved from the principal peak by overlay of the UV trace with the normalized polarimetric trace, as has been demonstrated for ephedrine [22]. Assuming that peak broadening is minimal, a pure enantiomer will exhibit a trace that is of the same shape on both the UV and polarimetric detectors. If two components of differing optical rotation or UV absorbance are imcompletely resolved, differences in the polarimetric and UV peak shapes will be observed. No such peak inhomogeneity was noted for nicotine.

Fig. 2 is a graph of the response of the ChiraMonitor to various loadings of (*R*)-

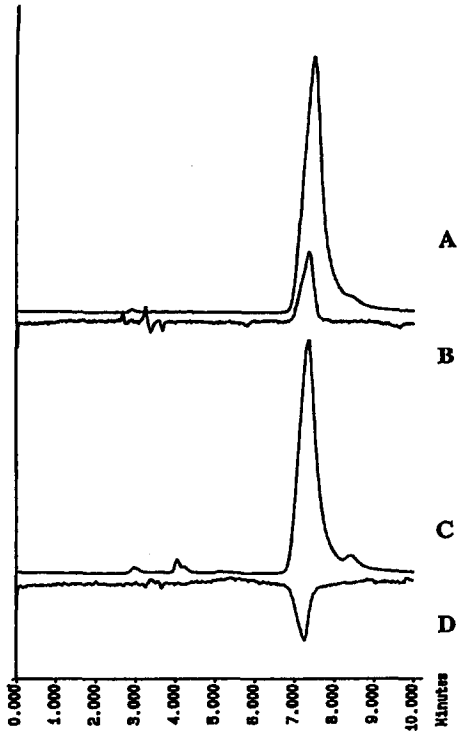

Fig. 1. Comparison of UV and ChiraMonitor response of *R*-(+)- and *S*-(−)-nicotine separated on PLRP-S. Separation was performed on a 5 μm, 25 × 0.46 cm I.D. PLRP-S reversed-phase column using water–acetonitrile–2-propanol (78:22:1) at 1 ml/min as the mobile phase. The water was buffered to pH 7.2 with 0.2% triethylamine phosphate. UV detection was at 276 nm. The upper traces are (A) UV and (B) ChiraMonitor response to *S*-(−)-nicotine. The lower traces are (C) UV and (D) ChiraMonitor responses to *R*-(+)-nicotine. The horizontal axis is retention time in min, while the vertical axis is response in arbitrary units.

and (*S*)-nicotine. Separations were performed as in Fig. 1. Fig. 2 demonstrates that the response of the ChiraMonitor is essentially linear with concentration up to a loading of 200 μg. Above that loading, there are deviations from linearity. The detection limit, defined as four times the peak-to-through amplitude of the noise, was determined to be about 12 μg.

Fig. 3 shows the specific optical rotations of chromatograms of mixtures obtained by the mixture of solutions of *R*-(−)-nicotine and *S*-(+)-nicotine. Separations were performed as in Fig. 1. Loading was about 200 μg. The on-line polarimeter exhibited a coefficient of variation of about 2.1% in the value of the rotation, and analysis of variance indicated that the percent standard error was 0.7% at the mean to 1.0% at the extreme. Therefore, the *purity* of an unknown sample can be determined to ± 0.5% or less by comparison with the calibration curve. The precision, of course, depends on the number of data points used to construct the calibration curve as well as the number of sample replicates, and would be lower if fewer data points were used. For the static polarimeter, an average coefficient of variation in the dupli-

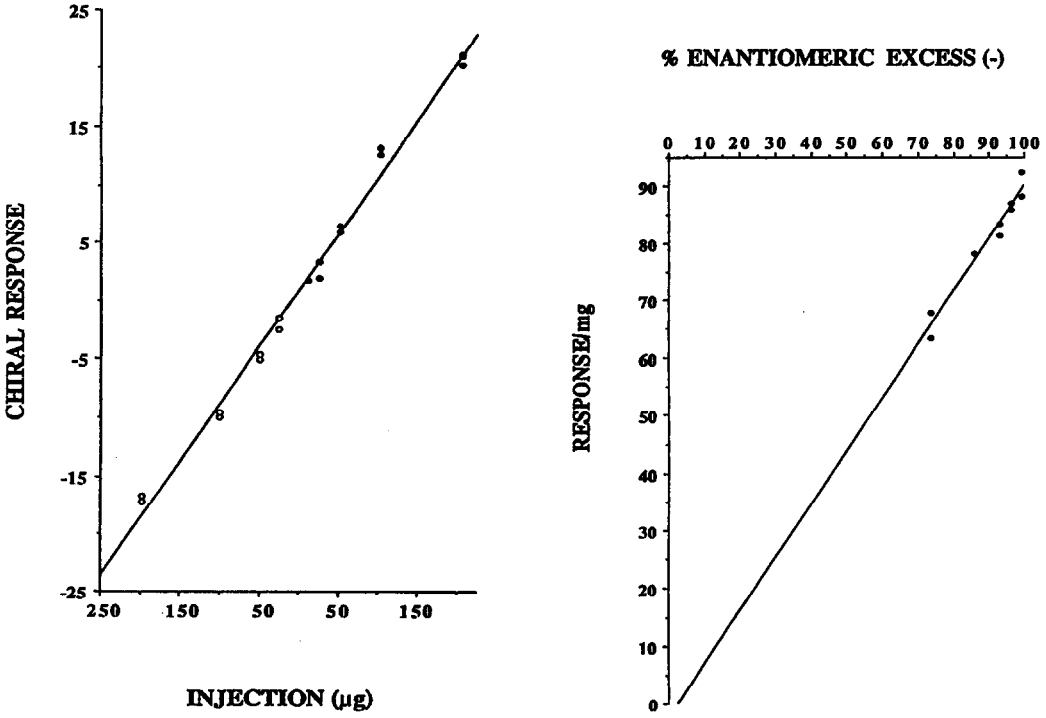

Fig. 2. Linearity of ChiraMonitor response to *R*-(+)- and *S*-(−)-nicotine. Separation conditions are as in Fig. 1. The vertical axis is the chiral peak height in arbitrary units. The horizontal axis to the right of zero is the loading of *S*-(−)-nicotine in units of µg, while the horizontal axis to the left of zero is the loading of *R*-(+)-nicotine in units of µg. ○ = *R*-(+)-nicotine, ● = *S*-(−)-nicotine.

Fig. 3. Spiking of *S*-(−)-nicotine with *R*-(+)-nicotine. Mixtures of *S*-(−)- and *R*-(+)-nicotine were prepared so that the total amount of nicotine was approximately 10 mg/ml. Then, 20 µl of each of these mixtures was injected onto the chromatographic system described in Fig. 1. The ChiraMonitor response divided by the loading is graphed on the vertical axis, while the enantiomeric excess is graphed on the horizontal axis. The mixtures presented on the graph represent duplicate injections of enantiomeric excesses of 99.1, 96.5, 93.0, 86.1 and 73.5% of *S*-(−)-nicotine. Reproduced, with permission from *American Laboratory* [22].

cate values of the observed rotation of 0.4% was observed. Analysis of variance indicated that the percent standard error in rotation increased from 0.2% at the mean to 0.4% at the extreme.

CONCLUSIONS

On-line polarimetry has been shown in the present work to be over 1000 times as sensitive and almost as precise as conventional polarimetry in the determination of the enantiomeric purity of nicotine. Several apparently optically inactive minor components, amounting to about 5% of the total were resolved from the nicotine peak by reversed-phase chromatography. Overlay of the UV chromatogram with a normalized ChiraMonitor trace indicated that no other component co-eluted with nicotine.

On-line polarimetry can be regarded as inherently more accurate than conventional polarimetry because minor components may be resolved chromatographically. Peak inhomogeneity, *i.e.* the presence of an incompletely resolved minor component may be readily detected if either the UV spectrum or the optical rotation differ from the those of the principal component.

It has been difficult to separate the enantiomers of nicotine by chiral chromatography [13–15], requiring the development of alternative means of quantitation. The *R*-(+)- and *S*-(−)-isomers differ in both efficacy and toxicity, making precise determination of optical purity desirable. The sensitivity of the ChiraMonitor was found to be several decades less than would be desired for pharmacological analysis of nicotine in blood and tissue, but adequate for determinations from urine. The greater specificity of polarimetric detection (relative to UV detection) makes it appropriate for complex samples, such as nicotine formulated for administration. On-line polarimetric determination of nicotine was rapid, requiring only about 10 min per run; sensitive, requiring only 200 μg of sample; and precise, permitting quantitation to about ± 0.5% purity.

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