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(2-Naphthoxy)acetyl chloride, a simple fluorescent reagent

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Abstract

In continuing the search for fluorescent reagents for analytical derivatization in chromatography, we found a simple chemical, (2-naphthoxy)acetyl chloride, with potential fluorophore/chromophore characteristics for the highly sensitive detection of analytes with an amino function. The reagent has an auxochrome (a substituted alkoxy moiety) attached to the fluorophoric/chromophoric naphthalene system, resulting in favorable spectrophotometric properties. The reagent can be easily prepared from (2-naphthoxy)acetic acid and has been used in organic synthesis; it is initially introduced as a fluorescent reagent to derivatise amantadine and memantine (amino pharmaceuticals) as model analytes. The resulting naphthoxy derivatives of the drugs can be analyzed at sub-μM levels by HPLC with fluorimetric detection (excitation wavelength 227 nm, emission wavelength 348 nm). Application of the reagent to the fluorimetric derivatization of important biological amines for sensitive detection can be expected.

Keywords: Derivatization, LC; Naphthoxyacetyl chloride; Amantadine; Memantine

1. Introduction

For improving detection sensitivity, analytical derivatization coupled with chromatography has been widely used in chemical, biomedical, environmental, and other analytical sciences [1–4]. Using chemical derivatization, a responsive label such as a fluorophore, chromophore or electrophore can be incorporated into a target analyte for sensitive analysis. We have developed several chemically removable sulfonate reagents for the highly sensitive analysis of carboxylic acids in biosamples by LC [5] or GC [6]. In a continuing search of derivatizing reagents for analytes with amino function, we found an interesting chemical (2-naphthoxy)acetyl chloride (NAC). NAC has an auxochrome attached to the chromophoric (fluorophoric) naphthalene, leading to its favorable absorption in spectrophotometric analysis. In fact, numerous reagents have been developed and documented for labeling amino analytes with fluorophores of acridine, benzofuran, coumarin, fluorene, fluorescein, naphthalene, quinoline, quinoxaline, and pyrene [7,8], reflecting on the ubiquitous existence of many important amino analytes which need to be analyzed at trace levels. Among the naphthalene chromophores, NAC is structurally simple and can be economically prepared from (2-naphthoxy)acetic acid. NAC has been used in organic synthesis [9–11] and is initially introduced here as a fluorescent reagent for the sensitive analysis of amantadine and memantine as model analytes

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which are amino pharmaceuticals which lack a chromophore.

2. Experimental

2.1. Chemicals and solutions

Amantadine (AT) and memantine hydrochloride (MT) (Sigma, St. Louis, MO, USA), 9,10-diphenylanthracene (as internal standard, I.S.) (TCI, Tokyo, Japan), (2-naphthoxy)acetic acid (Aldrich, Milwaukee, WI, USA), thionyl chloride, toluene, hexane, methanol, tetrahydrofuran and triethylamine (Tedia, Fairfied, OH, USA) were used without further treatment. Other chemicals were of analytical-reagent grade. Solutions of AT and MT were prepared by dissolving the appropriate amounts of the respective compounds in triethylamine (5 m*M*) in toluene. Solutions of NAC and I.S. were prepared in toluene. Distilled water purified with an Ultrapure R/O water system (Millipore, MA, USA) was used for all aqueous solution.

2.2. LC conditions

A Waters LC system with a model 1515 pump, a Model 717 plus autosampler and a Model 474 scanning fluorescence detector and a Breeze data system were used. A Merck Purospher Star RP-18e (125×4 mm I.D., 5 μm) and a mixed solvent of methanol–tetrahydrofuran–water–triethylamine (75:10:15:0.02, v/v) at a flow-rate of 1.0 ml/min were used. The column elutes were monitored fluorimetrically (excitation wavelength 227 nm; emission wavelength 348 nm).

2.3. Synthesis of (2-naphthoxy)acetyl chloride

(2-Naphthoxy)acetic acid (2.02 g, 10 mmol) and thionyl chloride (8 ml, 110 mmol) were placed in a 25-ml reaction flask attached to a reflux condenser with an NaOH-filled tube. The mixture was magnetically stirred and refluxed at 80 °C for 2 h. The reacted solution was concentrated in a rotary evaporator. The residue obtained was crystallized in *n*-hexane and yellowish white needle crystals were obtained; m.p. 96–97 °C (uncorrected) similar to that reported in Ref. [11]. Electron impact ionisation (EI) MS: m/z 220$(M)^+$, m/z 222$(M+2)^+$ and m/z 127$(C_{10}H_7)^+$ (naphthyl moiety).

2.4. Derivatization procedure

A 300-μl aliquot of AT and MT solution (10 μ*M* each in 5 m*M* of triethylamine in toluene) was added to a 25-ml screw-capped test tube containing 100 μl of I.S. solution and 100 μl of NAC solution. The reactants were shaken at 30 °C for 6 min. After derivatization, 250 μl of methanol was added and the solution was further reacted at 30 °C for 8 min to inactivate excess NAC. The methanol-treated solution was subjected to HPLC analysis (20 μl).

3. Results and discussion

NAC can be readily prepared from the commercially available chemicals (2-naphthoxy)acetic acid and thionyl chloride by a simple one-step reaction. As expected, NAC is reactive to nucleophilic analytes such as primary amines (amantadine and memantine), secondary amines (piperidine and diethylamine), aromatic amine (aniline), primary alcohol (*n*-propanol), secondary alcohol (2-propanol), tertiary alcohol (haloperidol), phenol and thiophenol by TLC screening test (data not shown).

Amantadine and memantine are widely used as an antiparkinsonism and a skeletal muscle relaxant, respectively. They are alicyclic amines without chromophores (Fig. 1) and selected as model analytes for analytical derivatization with NAC.

3.1. Some characteristics of NAC

NAC has an auxochrome (substituted alkoxy) attached to the chromophoric (fluorophoric) naphthalene (Fig. 1), resulting in good spectrophotometric properties. No amino or hydroxy auxochrome is directly linked to the chromophoric naphthalene system. That could be favorable since the chromophoric properties of a derivative from a chromophore with ionizable groups are generally supposed to be affected by a pH change in an analytical system (such as mobile phase) without controlled pH. NAC is a simple reagent that can be

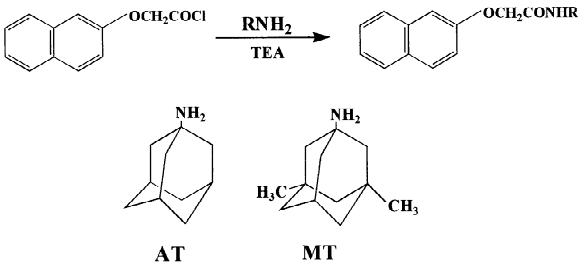

Fig. 1. Simplified reaction scheme for the derivatization of amantadine (AT) and memantine (MT) with (2-naphthoxy)acetyl chloride (NAC). RNH_2 as a functional form for AT or MT and TEA for triethylamine.

synthesized as a solid for easy storage. NAC is sensitive to moist air, but it can be kept in a brown desiccator for several months without a change in appearance. NAC is highly reactive to amino compounds, as reflected in its fast derivatization of AT and MT (Section 3.2).

3.2. Optimization of the derivatization

For adopting a solvent that can be used both as a reacting solvent for derivatization and an extracting solvent for separating AT and MT from biosample, water insoluble toluene was studied as a reacting solvent in optimizing the derivatization of AT and MT (3 nmol each i.e. a solution of 10 μ*M*, 300 μl). The main parameters in the derivatization procedure (Section 2.4) were varied to evaluate their effects on the derivatization (while maintaining a constant temperature of 30 °C). The effects of various amounts of NAC (10–1000 nmol i.e. 0.1–10 m*M*, 100 μl) on the derivatization indicate that the equilibrium formation of the AT and MT derivatives is attainable using NAC at amounts $\geqslant$50 nmol (0.5 m*M*, 100 μl); and the effects of derivatization times (0.5–10 min) on AT and MT show that the plateau formation of the derivatives is attainable in 30 s. Reaction times $<$30 s were not studied further. It seems that NAC is highly reactive to AT and MT. Considering derivatization of AT and MT in a real sample (such as plasma in Section 3.6) coexisted with various nucleophilic matrixes, sufficient time (6 min) was selected for the derivatization of AT and MT at 30 °C.

3.3. Stability of the derivative

The stabilities of AT and MT derivatives at room temperature after completing the derivatization procedure (Section 2.4) were studied over a period of 48 h. No significant change in the peak-area ratios of AT or MT to the I.S. were found, indicating that the derivatives are sufficiently stable for the time required for LC analysis.

3.4. Analytical calibration and precision

Based on the suitable derivatization conditions, the derivaitzation procedure for AT and MT was formulated as in Section 2.4. The quantitative applicability of the method for AT and MT was evaluated at five different concentrations of AT or MT over the range of 0.5–10 μ*M* (each in 300 μl of reference solution). The calibration graphs were established with the peak-area ratios of the AT or MT derivative to I.S. as the ordinate (y) vs. the concentration of AT or MT (μ*M*) as the abscissa (x). The linear regression equations obtained are $y = (0.278 \pm 0.005)x + (0.052 \pm 0.001)$ with a correlation coefficient (r) 0.999 ($n = 5$) for AT analysis and $y = (0.292 \pm 0.007)x - (0.009 \pm 0.0003)$ with $r = 0.999$ ($n = 5$) for MT analysis. The detection limit ($S/N = 3$, sample size 20 μl) of AT or MT is about 0.15 μ*M* based on AT or MT in the reference solution (300 μl).

The intra-day precision (relative standard deviation, RSD) and accuracy (relative error, RE) of the method were studied based on the peak-area ratios for the analysis of AT and MT each at three levels, 1.0, 4.0 and 8.0 μ*M*. The results ($n = 5$) indicate that the RSD and RE for AT were below 2.6% and 1.4%, respectively, and for MT were below 2.5% and 2.0%, respectively.

3.5. Mass spectral analysis of the derivatives

The derivatives of AT and MT were synthesized by scaling up the amount of AT or MT (0.1 mmol) with a similar procedure to that indicated in Section 2.4 without the addition of I.S. The purified derivative was examined by fast atom bombardment (FAB) MS (VG Quattro 5002 mass spectrometer with an acceleration energy of 10 kV, using nitrobenzyl

alcohol as a matrix). The mass spectra obtained exhibited diagnostic peaks: for the AT derivative, m/z 334 $(M-H)^+$ and a base peak m/z 135 $(AT-NH_2)$; and for the MT derivative, m/z 362 $(M-H)^+$ and m/z 163 $(MT-NH_2)$. The retention times of the synthesized AT and MT derivatives are identical to those from the derivatization of the reference solutions of AT and MT.

3.6. Application

Preliminary analysis of AT and MT spiked in plasma (from a normal 25-year-old male volunteer) was studied using a similar derivatization procedure (Section 2.4) after extraction of AT and MT from plasma with toluene. Toluene was used both as a reacting and extracting solvent. This can avoid the additional time and effort required when using different solvents for extraction and derivatization [12–14]. The simple protocol for the extraction and derivatization of AT and MT is as follows: a normal plasma (300 μl) was spiked with AT and MT (3 nmol each in 50 μl of 0.1 *M* HCl). After mixing, the spiked plasma was alkalified with NaOH (2 *M*, 50 μl) and then extracted with toluene (400 μl). An aliquot (300 μl) of the toluene layer was transferred to a 25-ml screw-capped test tube and subjected to the derivatization of AT and MT as described in Section 2.4. The reacted solution was used for LC analysis. A typical chromatogram for the analysis of AT and MT spiked in plasma is shown in Fig. 2A. No significant interference from the plasma blank was found (Fig. 2B).

The quantitative applicability of the method for AT and MT spiked in plasma at five different amounts of AT over the range of 0.3–3 nmol was tested. The calibration graphs were established similar to those in Section 3.4, using y for the peak-area ratios of AT or MT derivative to I.S. but x (nmol) for the amount of AT or MT spiked. The linear regression equations obtained are $y=(0.916\pm0.004)x+(0.056\pm0.004)$ with an $r=0.999$ ($n=5$) for AT, and $y=(0.957\pm0.054)x-(0.078\pm0.001)$ with an $r=0.999$ ($n=5$) for MT. The intra-day precision and accuracy are shown in Table 1, based on the analysis of AT and MT each at three levels of 0.6, 1.2, 2.4 nmol. The results indicate that the RSD and RE values are all below 3.4%. Therefore, the method could be applicable for the pharmacokinetic study of AT and MT in plasma. The I.S. used can be replaced if an alternative chemical with similar structure to AT or MT derivative is available.

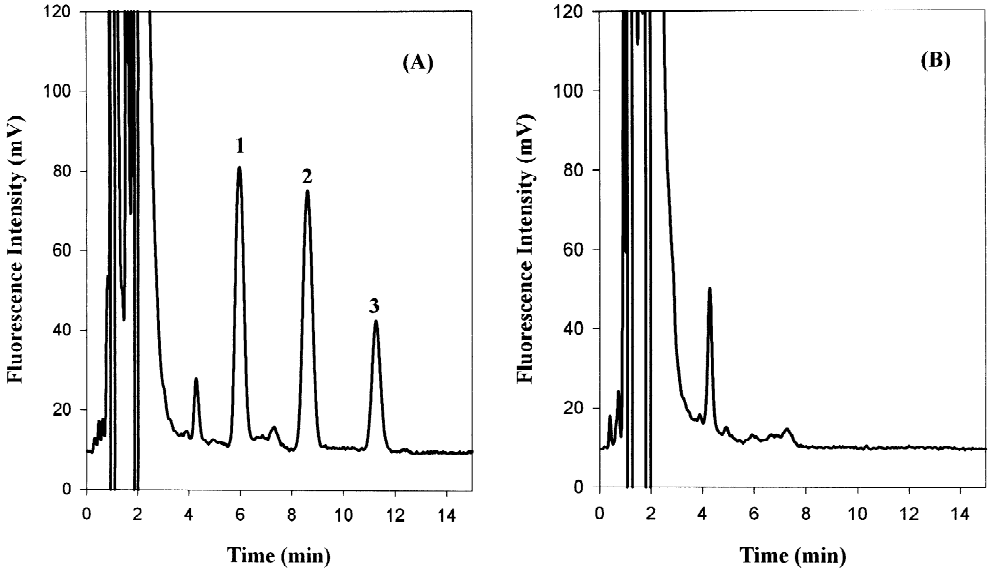

Fig. 2. Liquid chromatograms for amantadine and memantine in plasma derivatized with NAC (A) and plasma blank (B). Peaks: 1=AT derivative; 2=MT derivative and 3=I.S. For LC conditions see Section 2.2.

Table 1
Analytical results for AT and MT spiked in plasma

	Amount spiked[a] (nmol)	Amount found (nmol)	RSD[b] (%)	RE[b] (%)
AT	0.6	0.58±0.019	3.3	−3.3
	1.2	1.17±0.011	0.9	−2.5
	2.4	2.45±0.071	2.9	2.1
MT	0.6	0.62±0.007	1.1	3.3
	1.2	1.18±0.011	0.9	−1.7
	2.4	2.40±0.021	0.9	0.0

[a] Normal plasma (300 μl) was spiked with varied amount of AT or MT in 0.1 *M* HCl (50 μl).
[b] RSD and RE stand for relative standard deviation and relative error, respectively ($n=5$).

In conclusion, a simple, economic, and highly sensitive fluorescent reagent (2-naphthoxy)acetyl chloride was introduced for the first time to the fluorimetric liquid chromatographic determination of the basic drugs AT and MT at sub-μ*M* levels. The resulting derivatives of AT or MT bear an auxochrome attached to the chromophoric (fluorophoric) naphthalene system leading to a compound suitable for UV or fluorescent spectrophotometric detection. Application of the reagent to the trace analysis of various biological/pharmaceutical analytes with amino function is being studied.

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