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Determination of residual amines used in bulk drug synthesis by pre-column derivatization with 3,5-dinitrobenzoyl chloride and high-performance liquid chromatography

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Abstract

A rapid, simple and general method for determining residual amines used in bulk drug manufacturing is described. The method uses a modified Schotten–Baumann reaction to prepare the 3,5-dinitrobenzoyl derivatives of primary and secondary amines. Unlike previously reported procedures, the method does not require the isolation of the derivative and the reaction solution is chromatographed directly without further sample treatment. Application of the method to the determination of residual piperazine in 2-methylpiperazine (I), of 3-aminopyrrolidine in 3-(*tert*-butoxycarbonylamino)pyrrolidine (II) and *N*-(2-tetrahydrofuroyl)piperazine in 2-([4-(tetrahydro-2-furanyl)carbonyl]-1-piperazinyl)-6,7-dimethoxy-4-quinazolinamine, monohydrochloride, dihydrate (III) is described. Linearity was demonstrated with correlation coefficients ≥0.9999 for all examples. Assay precision (R.S.D. values) ranged from ±2.9% for the determination of 2.6% 3-aminopyrrolidine in (II) to ±11% for the determination of 0.21% *N*-(2-tetrahydrofuroyl)piperazine in (III). The accuracy of the determination ranged from 100.7 to 103.8%. The procedure is also used to determine the chiral purity of (3*S*)-(−)-(*tert*-butoxycarbonylamino)pyrrolidine.

Keywords: Derivatization, LC; Enantiomer separation; Pharmaceutical analysis; Amines

1. Introduction

Primary and secondary amines are used extensively as solvents and intermediates in the synthesis of pharmaceutically significant compounds. The quantitation of residual levels of these compounds is critical for minimizing potential manufacturing impurities and ensuring product integrity. The determination of residual amines directly by HPLC or GLC is difficult due to the low UV absorbance and poor chromatographic performance of this class of compounds, respectively. This necessitates the development of pre- or post-column derivatization schemes which must be capable of quantitating the residual amine of interest in the presence of a large excess of the bulk material. Our interests were in developing a general method which could be used to quantitate residual amine impurities in amine reagents, intermediates and in finished bulk drug materials. We report here a procedure based on the Schotten–Baumann reaction using pre-column derivatization with 3,5-dinitrobenzoyl chloride (DNBzCl) with an HPLC finish. The method was validated for the determination of residual piperazine in 2-

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methylpiperazine (I), for the determination of residual 3-aminopyrrolidine in 3-(*tert*-butoxycarbonylamino)pyrrolidine (II) and for determining residual *N*-(2-tetrahydrofuroyl)piperazine (THFP) in 2([4-(tetrahydro-2-furanyl)carbonyl]-1-piperazinyl)-6,7-dimethoxy-4-quinazolinamine (III). The same approach was used for determining the chiral purity of (3*S*)-(−)-(*tert*-butoxycarbonylamino)pyrrolidine by HPLC on a chiral stationary phase (CSP). This chiral assay was very useful in determining that an outside vendor was supplying us with material of sufficient chiral purity (>97% enantiomeric excess, ee) to carry on to bulk drug. The structures are shown in Fig. 1.

Pre-column benzoylations of primary and secondary amines using Schotten–Baumann conditions have been previously described for reversed-phase HPLC applications [1–4]. This approach is particularly useful in resolving the 3,5-dinitrobenzoyl (DNB) derivatives of asymmetric amines by HPLC [5–8]. In these examples the DNB derivatives are prepared, isolated from an aqueous matrix and chromatographed on normal-phase CSPs containing

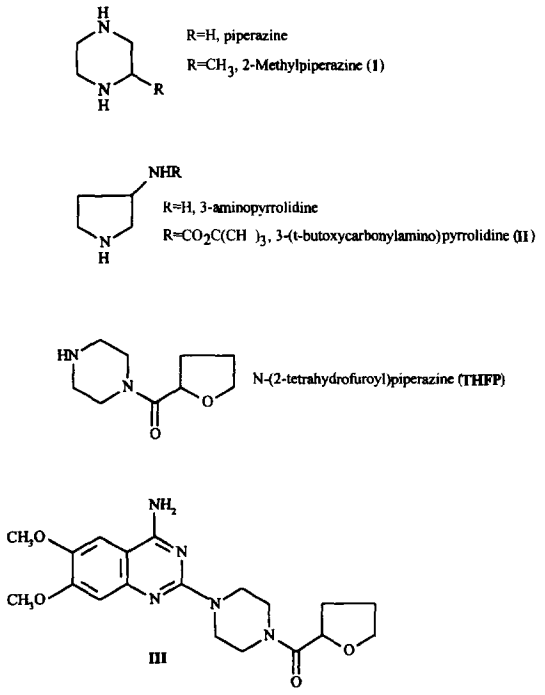


Fig. 1. Structures of the compounds studied.

π-donor sites. Solid-phase reagents containing the 3,5-dinitrobenzoyl moiety immobilized as an activated ester on a polymeric backbone have also been used for derivatizing achiral and chiral amines [9]. Novel GLC [10] and HPLC [11–13] CSPs have also been reported which resolve the DNB derivatives of asymmetric amines. Several general treatises dealing with the determination of amines via derivatization can be found in the literature [14–16]. Several recent publications have appeared which describe the derivatization and GLC determination of primary [17], secondary [18] and tertiary amines [19].

The procedure described here uses a simplified technique for determining residual amines by pre-column derivatization and HPLC. A homogeneous or solution procedure is used that rapidly gives a stable amide or diamide that can be chromatographed directly without any further sample treatment. Used previously for aminoglycosides [20], this approach allows for a high sample throughput with minimal analyst preparation time. The derivatization reagent, 3,5-dinitrobenzoyl chloride, was chosen because of its stability in aqueous environments and because the resulting amide derivatives have good UV sensitivity. The reaction generates 3,5-dinitrobenzoic acid, which can be readily manipulated in the chromatogram relative to the neutral amide product. The method was validated for determining residual levels of three distinctly different amines.

2. Experimental

2.1. Apparatus

The typical HPLC system consisted of a Model SP-8800 ternary pump and a Model 100 variable-wavelength UV detector (Thermo Separation Products, Santa Clara, CA, USA) with a Model C-R4A electronic integrator and a Model SIL-9A autosampler (Shimadzu, Kyoto, Japan). Achiral chromatographic separations were performed on either a Zorbax Rx-C_8 column (5 μm) measuring 25 cm×4.6 mm I.D. (Mac-Mod Analytical, Chadds Ford, PA, USA) or a Nucleosil C_{18} column (5 μm) measuring 15 cm×4.6 mm I.D. (Alltech Associates, Deerfield, IL, USA). The chiral separation of II was performed on a α-AGP column (5 μm) measuring 10 cm×4

mm I.D. equipped with a 10 mm×3 mm I.D. guard column (Regis, Morton Grove, IL, USA). All eluents were filtered through 0.45 μm nylon membranes prior to use (Alltech).

2.2. Reagents

Caution: 3,5-dinitrobenzoyl chloride is a corrosive lachrymator which is easily handled using normal laboratory precautions.

3,5-Dinitrobenzoyl chloride (98+%), (±)-3-aminopyrrolidine, dihydrochloride (99%) and 2-methylpiperazine were used as received from Aldrich (Milwaukee, WI, USA). Piperazine was used as received from Eastman Chemicals (Rochester, NY, USA). Acetonitrile was HPLC grade from EM Science (Gibbstown, NJ, USA). Potassium phosphate, dibasic and phosphoric acid (85%) were reagent grade from J.T. Baker (Phillipsburg, NJ, USA). THFP, III and (3*S*)-(−)-(*tert*-butoxycarbonylamino)pyrrolidine were prepared at Abbott Labs. (North Chicago, IL, USA).

2.3. Chromatographic conditions

The eluents used for the described assays are shown in Table 1. Isocratic elution was used to chromatograph the desired DNB derivatives. For the determination of THFP in III, additional acetonitrile and an increased flow-rate were added at the run's end to elute the highly retained DNB derivative of III. The 0.01 *M* potassium phosphate buffer, pH 7.3, was prepared by dissolving 3.5 g of dibasic potassium phosphate in 2 l distilled water followed by pH adjustment with phosphoric acid. The molarity was not corrected for the small amount of added phosphate. All chromatography was performed at ambient temperature. A summary of the chromatographic conditions used in the determinations is shown in Table 1.

3. Derivatization procedure

3.1. Determination of piperazine in I

Stock solutions of DNBzCl (8 mg/ml) and I (1 mg/ml) in acetonitrile were prepared in separate volumetric flasks. A 5 ml aliquot of the stock solution of I was combined with 5 ml of 1% phosphate buffer, pH 8, in a 100 ml volumetric flask. The solution was swirled and a 10 ml aliquot of the DNBzCl stock solution was added. The resulting solution was swirled occasionally at ambient temperature for 20 min, followed by dilution to volume with acetonitrile–distilled water (1:1). The piperazine content of this preparation was determined by the external standard method versus a 2.5 μg/ml (0.25%) piperazine standard derivatized similarly.

3.2. Determination of 3-aminopyrrolidine in II

The above procedure was followed except a stock solution of II was prepared at 1.7 mg/ml (free base concentration) in acetonitrile–distilled water (1:1) and a 3.0 ml aliquot of the DNBzCl stock solution was used for the derivatization reaction. A 3-aminopyrrolidine standard preparation of approximately 11 μg/ml (free base concentration) was derivatized in

Table 1
Summary of chromatographic conditions

Sample	HPLC column	Eluent[a]	Flow (ml/min)	Inj. volume (μl)	Detection (nm)
Piperazine in I	Nucleosil C_{18}	56:44	1.0	50	254
3-Aminopyrrolidine in II	Nucleosil C_{18}	65:35	1.0	20	230
THFP in III	Zorbax RX-C_8	82:18[b]	1.5[b]	50	254
II (chiral)	α-AGP	95:5[c]	0.5	10	230

[a] Binary mixture of distilled water–acetonitrile unless noted.
[b] At run's end (35 min) the ratio was changed to 10:90 (flow=2 ml/min) in 5 min and held for 35 min. The system was re-equilibrated at 82:18 (flow=1.5 ml/min) for 20 min prior to the next sample analysis.
[c] A binary mixture of 0.01 *M* K_2HPO_4 (pH 7.3)/acetonitrile.

the same manner. The same sample preparation was used to determine the enantiomeric purity of the *S*-isomer of II. A 1% standard of the DNB derivative of the *S*-isomer was used for quantitation.

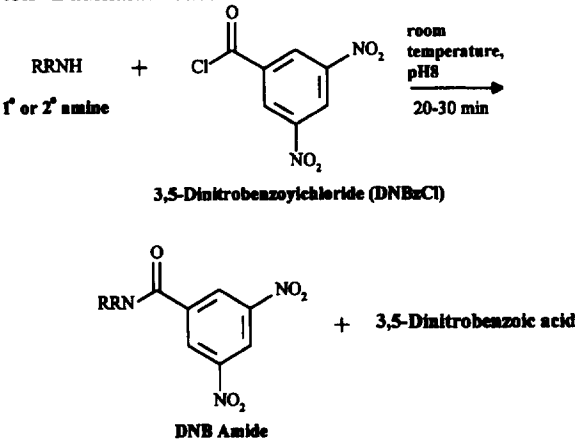
The identities of the DNB amide of II and the diamide of 3-aminopyrrolidine were confirmed by isolating material from a derivatization reaction run at a larger sale. The spectral properties (MS, ^{1}H-NMR, IR) were consistent with the proposed structures.

3.3. Determination of THFP in III

The procedure above was followed except the stock preparation of III was prepared at 5 mg/ml and 5% phosphate buffer, pH 8, was used. The THFP content was determined with a 5.0 μg/ml (1%) standard preparation prepared in the same manner.

4. Results and discussion

The residual amine contents of I, II and III (Fig. 1) was determined using the following modified Schotten–Baumann reaction:



Compounds I and II are reagents used as nucleophiles in the nucleophilic substitution of an aromatic halogen in the final steps of a drug synthesis (Fig. 2). The presence of piperazine in I leads to manufacturing impurities derived from piperazine displacement. Unlike 2-methylpiperazine where one nitrogen is sterically hindered, the piperazine-coupled product readily yields a highly retained dimeric

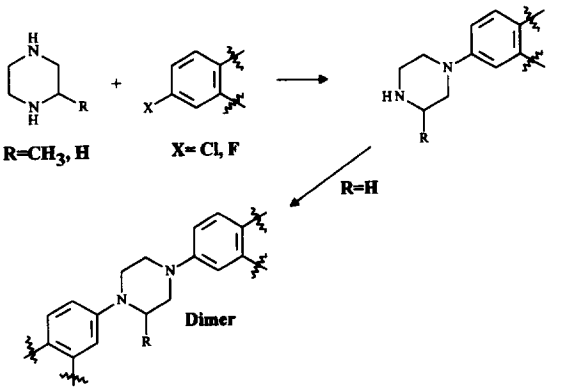


Fig. 2. Potential synthesis by-products from residual piperazine in I.

impurity. Likewise, residual 3-aminopyrrolidine in II can lead to competitive coupling between the ring nitrogen and the 3-amino group. Similarly, THFP is coupled with a quinazoline ring to give III. Shown in Fig. 3 are representative chromatograms of each sample preparation. As shown, the DNB derivatives are well resolved from the hydrolysis product, 3,5-dinitrobenzoic acid. The acid elutes at the solvent front and several minor impurities also present in the

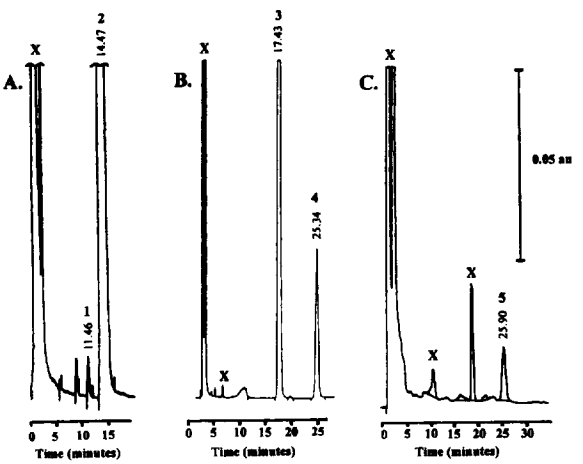


Fig. 3. Representative chromatograms of sample preparations. X=peaks from the derivatization reagent. Unlabelled peaks are unknowns. The chromatographic conditions are described in Table 1. (A) Residual piperazine in I determination; peak identities: 1=DNB-piperazine; 2=DNB-I. (B) Residual 3-aminopyrrolidine in II determination; peak identities: 3=DNB-II; 4=DNB-3-aminopyrrolidine. (C) Residual THFP in III determination; peak identity: 5=DNB–THFP.

Table 2
Derivatization linearity, x=concentration versus y=peak area counts

Compound	n[a]	Concentration (μg/ml)[b]	y-Intercept	Slope
Piperazine	4	1.1–7.9 (0.1–0.8%)	−512±176	5 280±35.5
3-Aminopyrrolidine	5	0.90–90 (0.1–10%)	−3090±11 200	29 000±249
THFP	6	3.8–250 (0.08–5%)	−2060±1480	4 640±12.7
II (chiral)	5	9.9–200 (0.5–10%)	−1000±458	5 470±4.54

[a] n=the number of data points used to generate the equation of the line.
[b] Free base concentrations; percentages of the sample preparation.

derivatization reagent do not interfere in the assay. Blank preparations were run to ensure a clear window for detection of the analytes of interest.

4.1. Reaction conditions

The conditions used for the derivatization reactions were optimized for reaction time, buffer concentration and DNBzCl concentration. The amide derivatives formed are soluble and stable in the diluted reaction mixture. Although the reaction is essentially complete after 5 min, the reaction times were extended another 15–25 min to maximize the hydrolysis of the excess reagent. The amount of active reagent remaining after partial dilution of a blank preparation was examined. Addition of II to this preparation gave less than 5% conversion to the DNB amide. This suggests adequate reagent hydrolysis prior to injection into the HPLC system.

4.2. Analytical data

The linearity of the derivatization reaction was examined for each residual amine assay. Samples of each amine at various concentrations were prepared and the solutions derivatized as described. In all cases the reaction was found to be linear over the concentration of interest. Plots of concentration (μg/ml, as the free base) versus peak area (counts) gave correlation coefficients greater than or equal to 0.9999 (Table 2). The data for the determination of enantiomeric purity of II were generated using the $S(-)$ isomer as the $R(+)$ enantiomer was not available in sufficient quantity.

The precision of the method was determined by two analysts using different instruments and columns. The samples chosen for the precision determination were known to contain residual amounts of the undesired amines. The accuracy of the method was determined by spiking samples of I, II and III with the appropriate amine and derivatizing the mixtures as described. For this experiment bulk materials were chosen which did not contain detectable amounts of residual amine. Recoveries were quantitative within the precision of the detection at addition levels appropriate to the anticipated levels of the residual amines. The precision and accuracy data are summarized in Table 3.

Table 3
Precision and accuracy data

	Piperazine in I	3-Aminopyrrolidine in II	THFP in III
Precision			
Mean (%)	0.086 (n=10)	2.6 (n=9)	0.21 (n=10)
Range (%)	0.081–0.092	2.4–2.7	0.18–0.23
R.S.D. (%)	±4.0	±2.9	±11
Accuracy			
(% Addition level)			
0.1	101.0 (n=2)	Not performed	101.0 (n=1)
0.5	100.7 (n=2)	Not performed	103.8 (n=1)
1.0	Not performed	102.2 (n=2)	103.7 (n=1)
2.0	Not performed	102.6 (n=2)	Not performed

4.3. Enantiomeric purity determination of II

The same sample preparation used for determining residual 3-aminopyrrolidine in II was used to assay the enantiomeric purity of the pure *S*-isomer. The DNB derivatives of the *R*- and *S*-isomers of II were successfully resolved on an α-AGP CSP. Attempts to directly determine the chiral purity of II without derivatization on Cyclobond I, Cyclobond III and Chiralpak WE(-) CSPs were unsuccessful. The preparation and chiral resolution of the DNB derivative of II has been previously described [21]. However, this derivative was chromatographed on a D-naphthylalanine Pirkle column which presumably required sample isolation prior to resolution on this normal-phase column. Shown in Fig. 4 are representative chromatograms of racemic material as well as a 1% *R*(+) isomer spike of an authentic sample. The enantiomers are well resolved (typical $R=1.6$) in less than 15 min. The pH of the phosphate buffer used in this separation is critical; at pH values below 7 the enantiomers begin to co-elute. No loss of resolution due to column degradation at pH 7.3 has been observed in over 100 h of operation. A sample of II spiked with approximately 1% *R*(+) enantiomer was derivatized and analyzed by two different analysts on different days using different instruments. The reproducibility of injection was $\pm 2.8\%$ with a mean of 1.1% and a range of 1.04–1.13% ($n=10$). The same procedure and chromatography can also be used to determine the enantiomeric purity of unprotected 3-aminopyrrolidine.

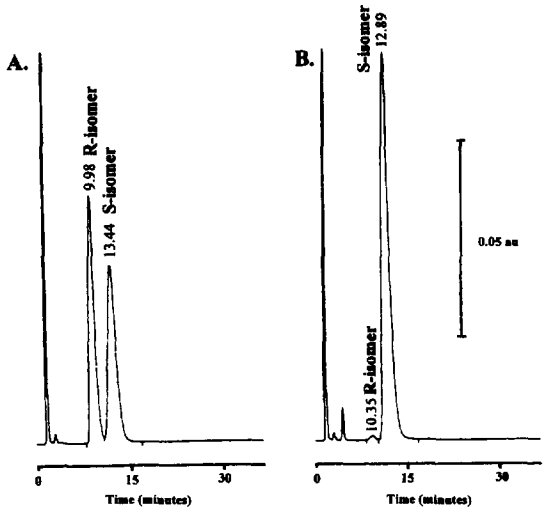


Fig. 4. Representative chromatograms for the determination of the enantiomeric purity of II. The chromatographic conditions are described in Table 1. (A) DNB derivative of racemic II. (B) DNB derivative of pure *S* isomer spiked with approximately 1% *R* isomer.

5. Conclusion

A rapid, simple and general method for determining residual amines has been described. The stable 3,5-dinitrobenzoyl amides prepared by a modified Schotten–Baumann procedure can be chromatographed directly without additional sample manipulation. Although not examined, the derivatization reaction has the potential to be fully automated which would further reduce the time necessary to carry out the analysis.

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