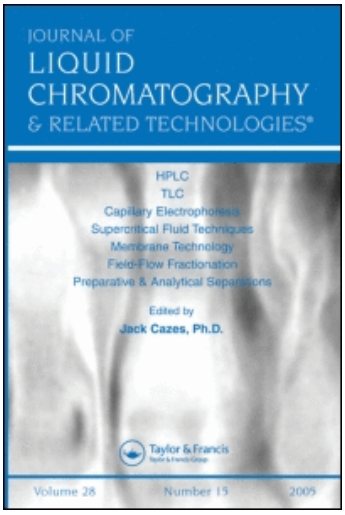

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Simultaneous Determination of ε-Caprolactam and ε-Aminocaproic Acid by Planar Chromatography

Ch. Sarbach[abc]; E. Postaire[b]; J. Sauzieres[c]

[a] Europlanaire, Chtenay-Malabry, Cedex, France [b] Department of Clinical Pharmacy and Biotechnique, School of Pharmacy, Chtenay-Malabry, Cedex, France [c] International Biopharmaceutical Services, Chevreuse, France

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SIMULTANEOUS DETERMINATION OF ε-CAPROLACTAM AND ε-AMINOCAPROIC ACID BY PLANAR CHROMATOGRAPHY

Ch. SARBACH[1,2,3], E. POSTAIRE[2], AND J. SAUZIERES[3]

[1]*Europlanaire*
47 Chemin de la Justice
92297 Châtenay-Malabry Cedex, France
[2]*School of Pharmacy*
Department of Clinical Pharmacy and Biotechnique
Rue JB Clément
92290 Châtenay-Malabry Cedex, France
[3]*International Biopharmaceutical Services*
BP 45
78470 St Rémy
lès Chevreuse, France

ABSTRACT

This paper describes the qualitative and quantitative determination of ε-caprolactam and ε-aminocaproic acid, which are potential contaminants of polyamid 6, by instrumental thin-layer chromatography (planar chromatography). A validation of the method is proposed. Detection was performed by photodensitometry in UV range at 200 nm for ε-caprolactam, and at 588 nm after derivatization by ninhydrin reagent for ε-aminocaproic acid. Correlation coefficients for calibration were found about 0.996 - 0.999. Repeatability results were included between 2.4% and 5.5%. Derivatization by Overpressured Derivatization (OPD) technique gave similar results as direct UV detection.

INTRODUCTION

Polyamid 6 (Nylon 6) is a widely used plastic material. The application fields of this polymer are multiple: car manufacture, textile, medical... The monomer of this material is ε-caprolactam. The synthesis way is well known : ε-caprolactam is first synthetized from hydroxylamine. In a further step, ε-caprolactam - cyclic molecule - is hydrolyzed in ε-aminocaproic acid, which is an aliphatic aminoacid. ε-aminocaproic acid is then polycondensed at 120° C, with elimination of water, to produce polyamid 6 (1). Thus, the two main potential impurities or degradation products of polyamid 6 are ε-caprolactam and ε-aminocaproic acid.

Polyamid 6 is described in a monograph of the French Pharmacopoeia X° edition. The limit level of ε-caprolactam required is 1% in the raw material. The assay method is gas-chromatography. However, all the studies performed in our laboratories were negative concerning the GC separation of ε-caprolactam and ε-aminocaproic acid. The method described in the pharmacopoeia's monograph allows only to quantify both products. The difficulty in the separation of the two components may be due to the possible condensation of ε-aminocaproic acid in ε-caprolactam in the injector or in the column (cf synthesis process above) due to the temperature.

The aim of this work is the simultaneous determination of ε-caprolactam and ε-aminocaproic acid separately, as potential contaminants of polyamid 6 used in the packaging of drugs in pharmaceutical industry, or in medical device. Indeed, the migration of ε-caprolactam into parenteral solutions packaged with polyamid has already been described. ULSAKER and TEIEN (2) showed the migration of ε-caprolactam from the envelope through the PVC barrier of an overwrapped PVC bag.

The technique tested is instrumental thin layer chromatography (planar chromatography).

EXPERIMENTAL

The tested sample was a methanolic solution containing 2 mg/ml of ε-caprolactam and 2 mg/ml of ε-aminocaproic acid. The stationnary phase was HPTLC silicagel without fluorescence indicator (Merck - Darmstadt-Germany).

The mobile phase was methanol/chloroform (50/50) in a saturated classical tank. A preliminary washing of the plate was carried out by development in the mobile phase; the aim was, on one hand to reduce the saturation time of the tank, on another hand to obtain a minimal solvent front after development.

Increasing amounts (2-10 mg) of ε-caprolactam and ε-aminocaproic acid were automatically streaked on 3 mm with a TLC-applicator AS30 (Desaga-Heidelberg-Germany).

The development distance was 50 mm from the line of streaks. The duration of development was about 15 min.

The post-chromatographic derivatization step was performed with ninhydrin/collidin reagent (3), by OverPressured Derivatization (OPD) technique (4,5), using a Derivabox° (Europlanaire-Châtenay-Malabry-France). This technique implies the use of a polymer foam which is first impregnated with the reagent, and secondly applied with pressure on the plate. The physical properties of the foam allow to reabsorb the excess of reagent at the pressure release.

The detection was carried out before and after derivatization, using a photodensitometer scanner CD60 (Desaga-Heidelberg-Germany), at a wavelength of 200 nm for ε-caprolactam (before derivatization) and 558 nm for ε-aminocaproic acid (after derivatization). The chromatograms were evaluated by integration of peak areas.

RESULTS AND DISCUSSION

Detection

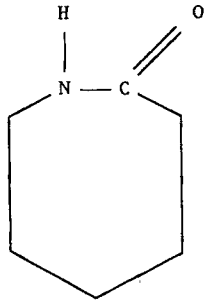
At 200 nm in direct UV detection :

Only ε-caprolactam was detected at 200 nm, because of the structure of this compound compared to ε-aminocaproic acid (fig 1) : the cycle and the amide group induce a light electronic delocalization, responsible for the absorption in the low wavelengths zone (fig 2).

On the contrary, the linear structure of ε-aminocaproic acid does not allow any UV absorption properties.

At 588 nm after OPD by ninhydrin/collidin reagent :

Ninhydrin/collidin reagent reacts with only primary amines, giving blue-violet coloured spots. Thus, it reacts with ε-aminocaproic acid,



ε - CAPROLACTAM

E-AMINOCAPROIC ACID

H_2N —— CH_2 —— CH_2 —— CH_2 —— CH_2 —— CH_2 —— COOH

FIGURE 1: Chemical formulas of ε-caprolactam and ε-aminocaproic acid.

which has presents primary amine structure, and not with ε-caprolactam, because of the secondary amine group. The spots of ε-aminocaproic acid gave a maximum at wavelength 588 nm.
Detection at 200 nm is the first mean to differentiate the 2 components.
Detection at 588 nm (visible) is the second suitable way of differentiation.

Chromatographic separation

The Rf determined for the 2 components were about 0,1 (5 mm of migration) for ε-aminocaproic acid, very polar, and about 0,93 for ε-caprolactam (fig 3). The separation is completely realized.
The suitable chromatographic separation is the third mean of differentiation of the 2 components.

Quantitation

Densitometric evaluation was performed laterally, thus only 1 chromatogram was registered to measure all the spots of each

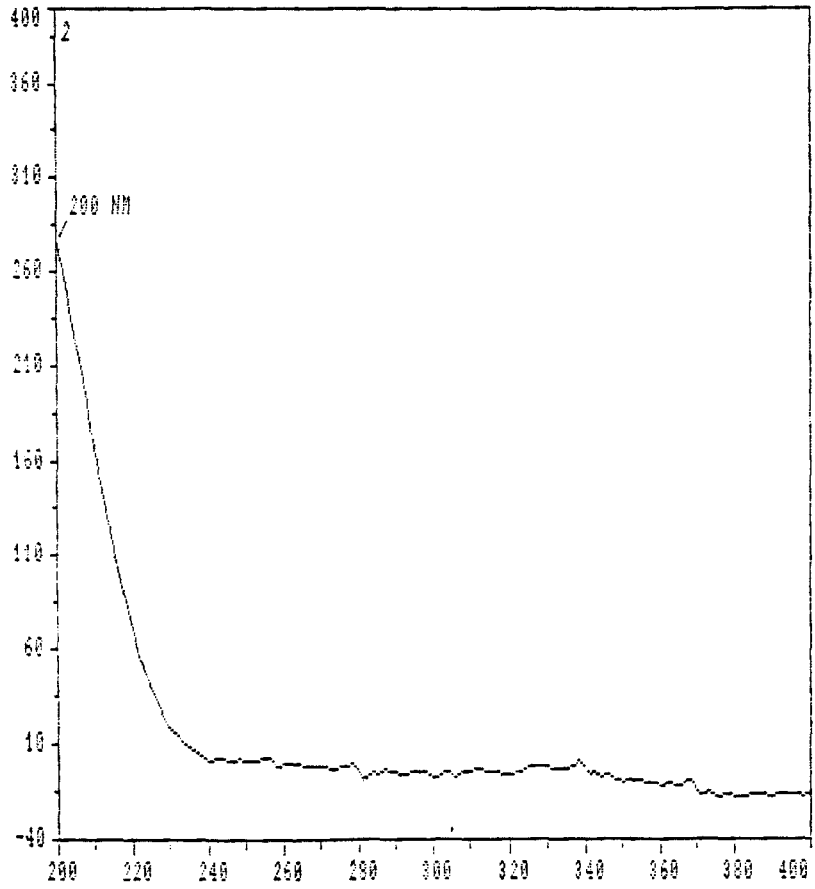


FIGURE 2: UV spectrum of ε-caprolactam.

component (fig 4). The main advantage of this method was the simultaneous integration of all the chromatograms, producing better results (same baseline). On another hand, the possible inaccuracy of the localization of streaks automatically applied is avoided. Indeed, even a little variation of distance between the spots, due to the application device, the detector, or the plate positioning can produce a significant difference.

Example : a variation of 0,1 mm of the distance between the spots at the application step induces a localization error of 1 mm at the detection of the 10^{th} spot.

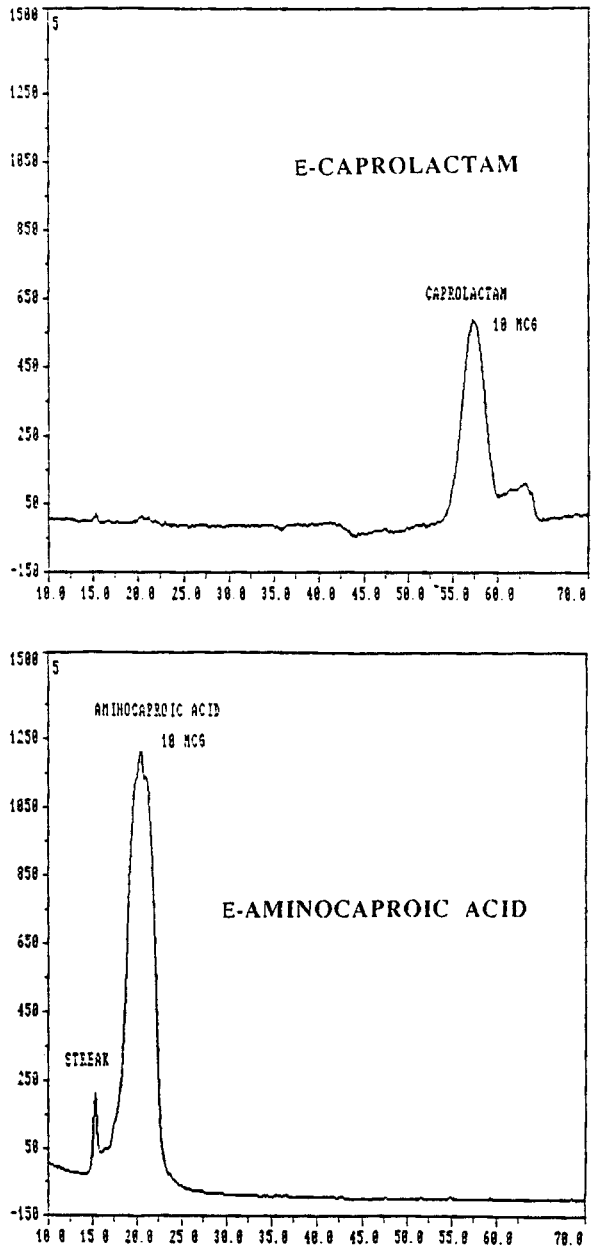


FIGURE 3: Chromatograms of ε-caprolactam and ε-aminocaproic acid.

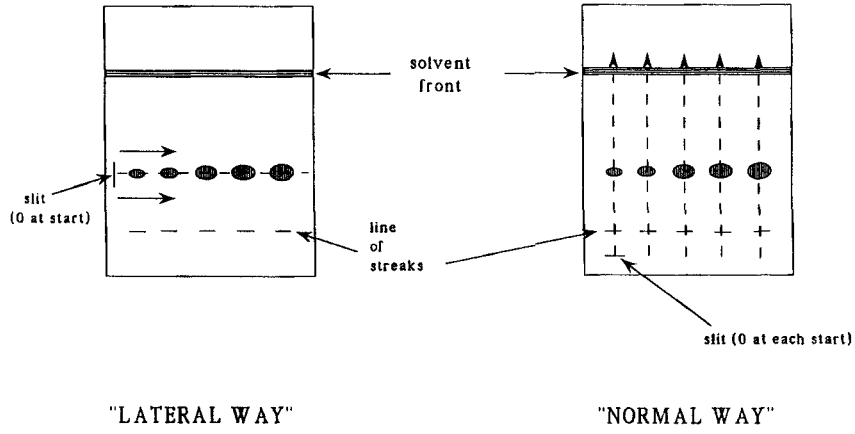


FIGURE 4: Detection modes.

However, before the "lateral" way of detection, it was necessary to perform a previous detection in the "normal" way, to evaluate the low Rf variations between the spots, and the size of the largest one (the highest calibration point), for the choice of the size and the co-ordinates of the slit position.

Calibration

The linearity in the calibration ranges of ε-caprolactam and ε-aminocaproic acid was checked. The chromatograms obtained for the two components are presented in the figure 5. The linear regression correlation coefficients (R^2) were 0,996 for caprolactam and 0,999 for aminocaproic acid (fig 6).

Detection limit

The detection limit for caprolactam was about 0,2 mg, and 0,02 mg for aminocaproic acid (fig 7). The effect of overpressured derivatization by ninhydrin-collidin reagent was to enhance the sensitivity of the detection, compared to direct UV detection of caprolactam.

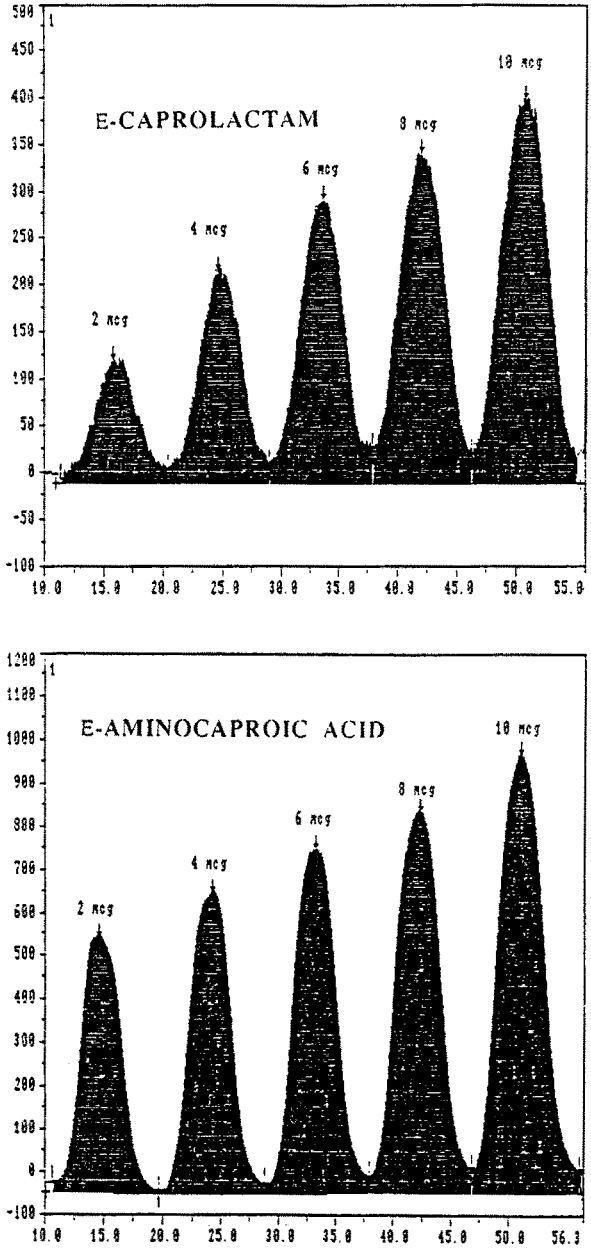


FIGURE 5: Chromatograms of calibration ranges of ε-caprolactam and ε-aminocaproic acid.

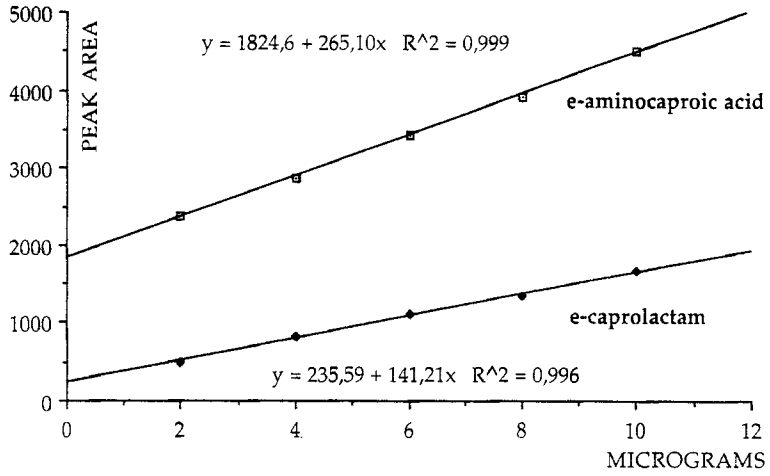


FIGURE 6: Calibration curves of ε-caprolactam and ε-aminocaproic acid.

Overpressured derivatization

OPD with Derivabox° apparatus was very easy-to-use, and produced good results (linearity, detection limit). After derivatization, it was necessary to keep the box air-tighted to avoid the drying of the foam, and to spare the reagent. Indeed, in case of drying, the homogeneity of the distribution of the reagent was not respected when impregnated again : so it was necessary to replace the foam. The manifestations of this "overloading" of the foam were founded on a plate containing 8 spots of the same solution, same volume (6 mg of ε-aminocaproic acid and ε-caprolactam), after development in the same conditions described above. We observed :

- increase of the mean coloration of the spots,
- inhomogeneity of the coloration of the spots : we observed the presence of yellow trails on the plate; the spots localized in these zones were much more coloured than the others.
- fringed outline of the spots.

The figure 8 shows the chromatograms of ε-aminocaproic acid in the repeatability study. The laterally detection mode was used. The chromatographic profile obtained with an "overloaded" foam is

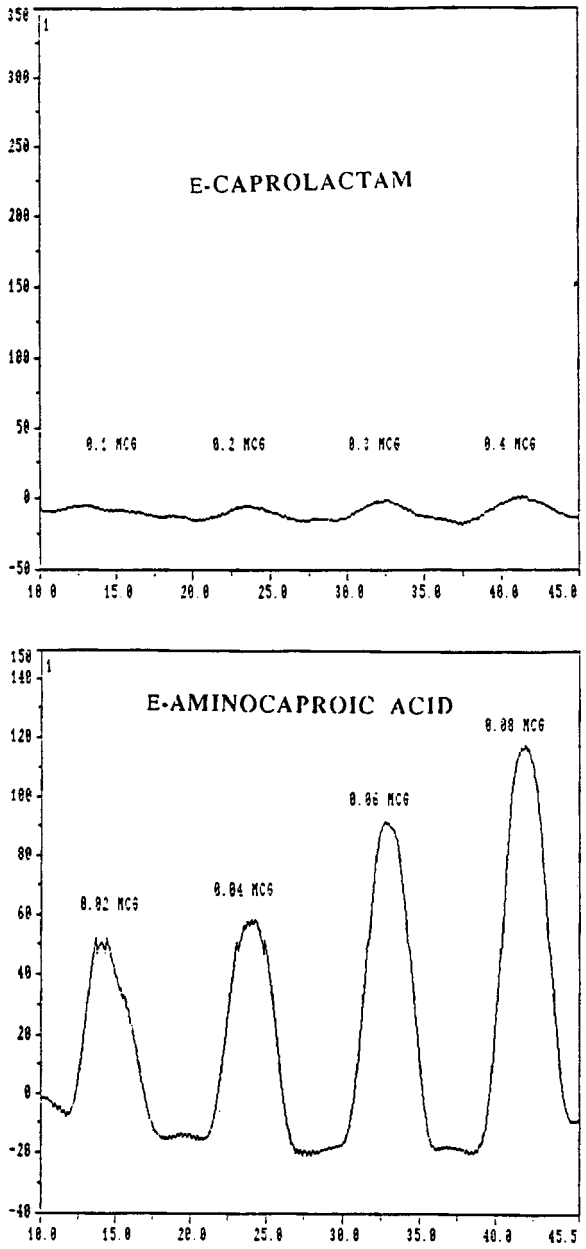


FIGURE 7: Detection limit of ε-caprolactam and ε-aminocaproic acid.

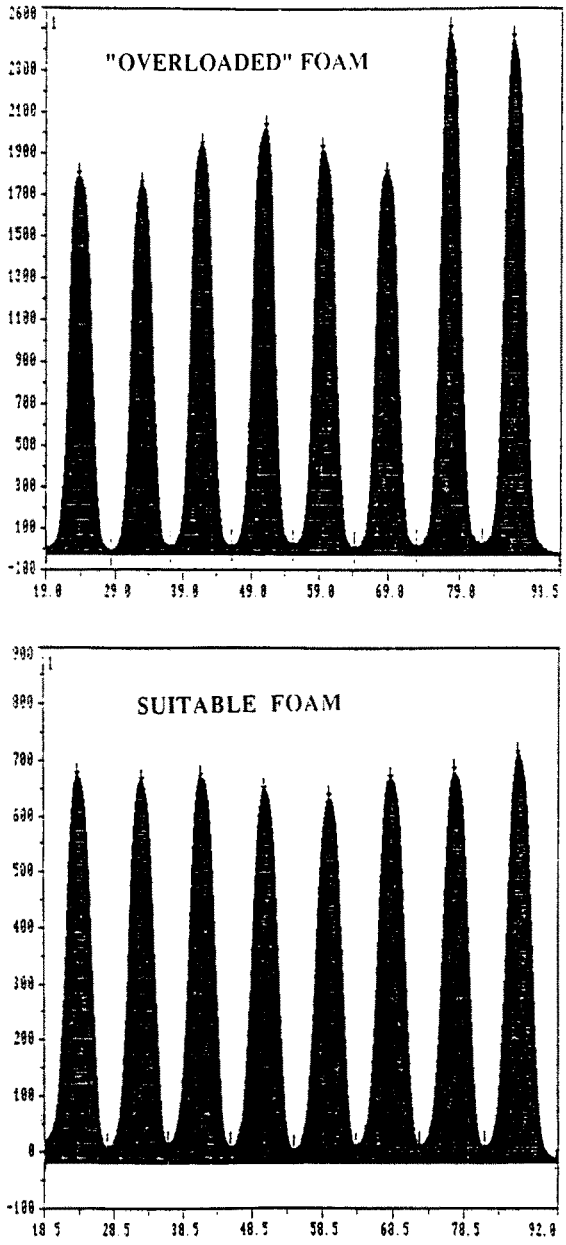


FIGURE 8: Compared chromatograms of ε-aminocaproic acid derivatized with "overloaded" foam or suitable foam.

TABLE 1: Compared repeatability results between UV detection, "overloaded" foam, and suitable foam.

n=8	caprolactam (200 nm)		aminocaproic acid "overloaded" foam	aminocaproic acid suitable foam
Mean of peak areas	995,0	950,9	7206	2856
RSD of peak areas	2,4%	5,5%	10,8%	3,7%

presented, compared to another plate in the same conditions using a new foam: the two spots on the left of the chromatogram obtained after derivatization with the "overloaded" foam were included in a yellow trail on the plate; the signal is therefore very higher.
The results of the absorbance and RSD values are presented table 1: these results show the importance of the quality of the foam in OPD. The RSD obtained with OPD performed in suitable conditions were similar than those obtained in direct UV detection.

In conclusion, the foams used in OPD must be removed and replaced in case of drying. Another case of replacement is the expiry date of the impregnating reagent.

CONCLUSION

Important improvements occured in planar chromatography during the last 10 years, and the field of applications grew rapidly.

The separated determination of ε-caprolactam and ε-aminocaproic acid is particularly interesting, in regard to their simultaneous presence as impurities of polyamid 6, and their difference of toxicity.

Gas chromatography is not suitable in this aim. Planar chromatography allowed to perform this dosage, and the method proposed was validated.

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