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# A convenient identification of carbamate-derived drug substances

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The “second identification” of drugs in pharmacies and hospital pharmacies affords the availability of easy to perform and robust tests. Besides determination of melting points and simple chromatography (TLC), reliable color and precipitation reactions are common techniques for this purpose. Preferentially, these reactions allow for the identification of typical functional groups in the drugs. Here we present a reaction for identification of carbamate-type drugs, based on the precipitation of barium carbonate upon treating the analytes with barium hydroxide solution at 80 °C. This method works well for carbamate drugs with noteworthy water solubility like carbachol, neostigmine bromide, and pyridostigmine bromide, and could be considered as a method for second identification of these drugs in pharmacopoeias and in Deutscher Arzneimittel-Codex/Neues Rezeptur-Formularium (DAC-NRF).

## 1. Introduction

„Substances for pharmaceutical use“ in the sense of the European Pharmacopoeia (Ph. Eur.) are substances that are used as active ingredients or excipients for the production of medicinal products for human or veterinary use (Ph. Eur. 9.0). Where medicinal products are manufactured using these substances, the user has to control the identity of all ingredients, typically using the methods described in the section „Identification“ in the individual Ph. Eur. substance monographs. Where this section contains subdivisions entitled ‘First identification’ and ‘Second identification’, the test or tests that constitute the ‘First identification’ may be used under all circumstances. The test or tests that constitute the ‘Second identification’ may be used in pharmacies or hospital pharmacies provided it can be demonstrated that the substance or preparation is fully traceable to a batch certified to comply with all the other requirements of the Ph. Eur. monograph. The identification tests of pharmacopoeias are not designed to confirm the chemical structures of the analytes in detail, rather they are designed to provide, with an acceptable degree of assurance, confirmation that the product conforms to the denomination given on the label of the purchased product.

As pointed out in detail in the EDQM „Technical guide for the elaboration of monographs“ (EDQM 2015), identification according to the pharmacopoeia is generally of a much more limited scope than the structural elucidation of an unknown substance, and the tests do not require more experimental effort than necessary for differentiating the substance in question from other pharmaceutical substances available in commerce. Whereas the ‘First identification’ typically requires rather complex instrumentation (IR, NMR, HPLC, GC), the ‘Second identification’ is characterized by three major techniques: determination of physical constants (melting point, optical rotation, relative density, refractive index), chemical reactions (colour or precipitation reactions), and chromatographic examination by TLC. A considerable number of commonly applied colour or precipitation reactions is listed in the Ph. Eur. general chapter „2.3.1. Identification reactions of ions and functional groups“.

Less common chemical reactions for identification are described in full in the individual monograph. In case more than one of these reactions are used for identification, each of these is to be chosen to demonstrate the presence of a different part of the molecule to be identified.

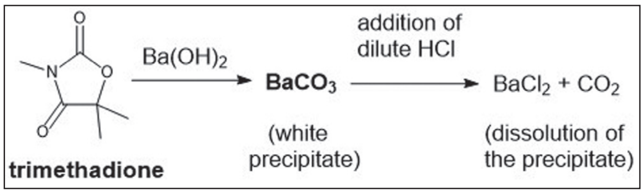
In the past decades, the ‘second identification’ received decreasing interest by the authorities of the pharmacopoeias of the industrialized countries, but very recently, the significance of this topic was appreciated by the EDQM in 2016, and a new „Second Identification Test (SIT) Working Party“ was established in order to revise existing and develop novel methods for chemical identification of substances for pharmaceutical use.

In Germany, the Deutscher Arzneimittel-Codex/Neues Rezepturformularium (DAC/NRF), a supplement to the pharmacopoeia published by an independent expert commission under the auspices of ABDA (Federal Union of German Associations of Pharmacists), publishes monographs which are being used as recognized standards by industrial manufacturers for the certificates of analysis of starting materials according to the Regulation on the Operation of Pharmacies (ApBetrO). Since 2007, DAC/NRF has published alternative identification test methods („Alternative Identifizierung“) of several hundred substances to simplify the incoming identification test of these goods by the pharmacy, without reducing the quality and safety. As in DAC/NRF it always is ensured that at least the identification can be performed in normally equipped laboratories in every pharmacy, chemical identification methods are of outstanding importance as well (DAC/NRF 2018).

In continuation of our work on the development of novel identification tests for drugs (Baumann et al. 2006) we now focused on the chemotype of drugs formally derived from carbonic acid, preferentially carbamates (synonym: urethanes), but also dialkyl carbonates and ureas. The carbamate group is an essential moiety in indirect cholinergics (physostigmine, neostigmine, pyridostigmine), but beyond this carbamates found broad application in medicinal chemistry in the past decades, and drugs and drug candidates for a broad spectrum of pharmacologic indications have been developed. The synthesis, chemical and metabolic stability and applications of carbamates have been reviewed recently (Ghosh and Brindisi 2015).

Consequently a general chemical identification of the carbamate would serve as a meaningful group reaction for this class of drugs. On the other hand, carbamate groups are widespread among drugs, but only few members of the single drug groups bear the carbamate (e.g. cefuroxime and cefoxitin among dozens of carbamate-free cephalosporins; carbimazole as the only marketed thyreostatic drug bearing a carbamate). In these cases an identification of the carbamate moiety would allow distinguishing one member of the respective class from the others.

Our investigations were inspired by identification test C of trimethadione (Ph. Eur.). Here, the drug, which is a polar, low-molecular aliphatic cyclic *N*-acylcarbamate is reacted with aqueous $Ba(OH)_2$ solution at room temperature, resulting in an almost immediate formation of a white precipitate, barium carbonate. Hydroxide ions from $Ba(OH)_2$ lead to hydrolysis of the carbamate, the formed carbonate ions are precipitated as the poorly soluble barium salt. The latter dissolves again after addition of hydrochloric acid under formation of readily soluble barium chloride and carbon dioxide gas (Scheme).



Scheme: Ph. Eur. identification test C for trimethadione.

## 2. Investigations and results

We submitted a considerable number of carbamate-type drugs to an analogous treatment. However, this investigation was expectedly handicapped by solubility issues. It was obvious that, in contrast to trimethadione, most of the drugs of interest do not have sufficient water solubility for allowing to perform the tests in aqueous solution, so that organic, water-miscible co-solvents were needed. On the other hand, the content of organic solvent could only be enhanced moderately, since otherwise the reagent barium hydroxide ($Ba(OH)_2$) would precipitate, ending up in false-positive results. Furthermore, in the final acidification step, precipitation of re-protonated analytes (or artifacts formed from them by base treatment) would disguise the re-dissolution of the barium carbonate precipitate. Orienting experiments revealed that the test solution would tolerate 50% ethanol. Consequently, polar test substances were investigated in aqueous solution, less polar substances in ethanol-water (1:1), whereas drugs that did not give clear 50 mg/mL solutions in ethanol-water (1:1) were not considered for further investigations.

**Table 1: Results of the barium hydroxide treatment of drugs**

| Drug | $Ba(OH)_2$ at 20 °C | $Ba(OH)_2$ at 80 °C | Treatment with HCl |
|---|---|---|---|
| **Type A carbamate drugs (investigated in aqueous solution)** | | | |
| Trimethadione | white precipitate | not performed | precipitate dissolves |
| Carbachol | no precipitate | white precipitate | precipitate dissolves |
| Cefoxitin sodium | no precipitate[a] | white precipitate | separated[b]; dissolves |
| Cefuroxime sodium | no precipitate | white precipitate[c] | separated[b]; dissolves |
| Neostigmine bromide | no precipitate | white precipitate | precipitate dissolves |
| Pyridostigmine bromide | no precipitate | white precipitate | precipitate dissolves |
| **Other type A drugs (investigated in aqueous solution)** | | | |
| Urea | no precipitate | no precipitate | not performed |
| Barbital sodium | no precipitate | cloudy solution | not performed |
| Phenobarbital sodium | no precipitate | white precipitate | separated[b]; dissolves |
| **Type B drugs (investigated in ethanol-water (1:1))** | | | |
| Barbital | no precipitate | no precipitate | not performed |
| Molsidomine | no precipitate | white precipitate[d] | precipitate dissolves[e] |
| Phenytoin sodium | no precipitate | white precipitate | separated[b]; dissolves |

[a] Solution turns yellow, but remains clear; [b] precipitate separated after centrifugation. [c] mixture turns orange; precipitate separated after centrifugation. [d] mixture turns yellow; [e] clear yellow solution results

Besides carbamates, we also investigated drugs bearing other functional groups that can formally undergo alkaline hydrolysis to give carbonate. Among these groups were dialkyl carbonates (the cephalosporin cefpodoxime proxetil and the glucocorticosteroid prednicarbate), (*N*-sulfonyl)ureas (urea, carbamazepine, and sulfonylureas), as well as heterocycles like barbiturates, nitrofurantoin, and phenytoin.

The investigated drugs are presented in the Figure Type A compounds showed significant water solubility, and could be analyzed in aqueous medium, type B compounds were sufficiently soluble in ethanol-water (1:1) for this test, whereas type C drugs were identified to be incompatible with the test conditions due to their poor solubility, and were not considered for further investigations.

Since first experiments revealed that only trimethadione gave a barium carbonate precipitate at room temperature, barium hydroxide treatment of drugs of type A and type B was also performed at 80 °C. Table 1 summarizes the results of these tests.

## 3. Discussion

The data shown in the Table clearly indicate the outstanding reactivity of trimethadione, since it is the only investigated drug that gives a barium carbonate precipitate at room temperature. This might be attributed to the *N*-acyl carbamate moiety. But this test can also be applied to water-soluble primary and tertiary carbamates. Primary carbamates can undergo alkaline degradation on two different pathways: anhydrous bases like alkoxides lead to release of isocyanate ion, whereas under aqueous conditions carbonate is the main product (Dittert and Higuchi 1963). However, these drugs give this test only after heating at 80 °C in a water bath for a few minutes. So this reaction represents a very reliable and easy to perform test for polar carbamates like carbachol, neostigmine bromide, pyridostigmine bromide, and molsidomine. In case the reaction mixtures turn intensively colored in the course of the reaction the final part of the test (visual test for re-dissolution of barium carbonate precipitate upon addition of hydrochloric acid) is problematic. We could demonstrate for cefoxitin sodium and cefuroxime sodium by means of centrifugation and separation of the precipitate (which readily dissolved upon addition of hydrochloric acid) that the reaction takes place very well. However, this centrifugation step renders this test unfeasible for application in second identification in pharmacies. Further, this test cannot be applied to carbamate drugs lacking the required solubility in aqueous or dilute ethanolic solution (e.g., carbimazole, efavirenz, zolmitriptan).

Attempts to extend this test to other functional groups that can in principle release carbonate upon alkaline treatment were of limited success: The investigated dialkyl carbonates (cefpodoxime proxetil, prednicarbate) had to be discarded due to solubility issues, the same holds for several urea derivatives (carbamazepine, glibenclamide, tolbutamide), whereas unsubstituted urea was found to be unreactive. In the barbiturate/hydantoin group, phenobarbital sodium and phenytoin sodium (type B drug, tested in ethanol-water mixture) in fact gave barium carbonate precipitates when heated to 80 °C, but with these analytes re-dissolution of the precipitate by direct addition of hydrochloric acid was accompanied by simultaneous precipitation of the re-protonated drugs (or artifacts formed from them with barium hydroxide). The initially formed precipitates were identified as barium carbonate after centrifugation and separation. But once again, due to the uncommon centrifugation step this procedure is not suitable for a second identification protocol. Interestingly, barbital sodium, in contrast to phenobarbital sodium, did not give a barium carbonate precipitate. So this test is not reliable in the class of barbituric acid derivatives.

A considerable number of drugs derived from carbonic acid can be converted into a barium carbonate precipitate by treatment with barium hydroxide at 80 °C. The re-dissolution of this precipitate upon addition of hydrochloric acid makes this test rather specific. This test works very well for hydrophilic carbamate-type drugs like carbachol, neostigmine bromide, and pyridostigmine bromide,

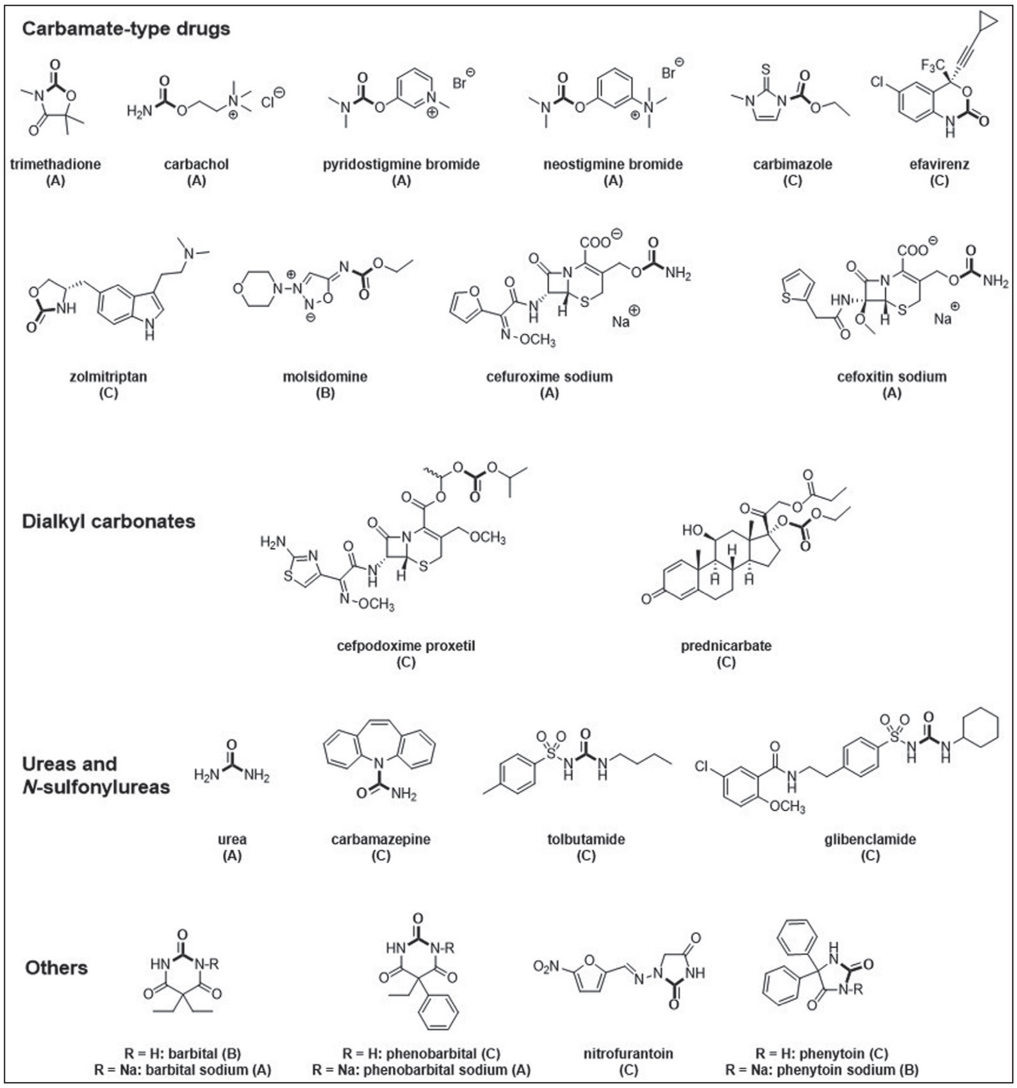


Fig.: Structures of the investigated drugs. In parentheses categorization on basis of solubility parameters: (A) suitable for performing the test in aqueous solution, (B) suitable for performing the test in ethanol-water (1:1), (C) not suitable for the test due to insufficient solubility in ethanol-water.

and should be considered as an alternative for second identification of these drugs. Limitations of this identification mainly arise from solubility issues, since a high water content in the reaction mixture is mandatory for this test.

## 4. Experimental

### *4.1. Reagents*

Barium hydroxide octahydrate p.a. was purchased from AppliChem (Darmstadt, Germany), hydrochloric acid (concentrated) from Brenntag (Essen, Germany), anhydrous ethanol from BrüggemannAlcohol (Lutherstadt Wittenberg, Germany). Drugs: trimethadione (Sigma-Aldrich Chemie, Munich, Germany), carbachol (TCI Europe, Zwijndrecht, Belgium), carbamazepine (Sigma-Aldrich Chemie), carbimazole (TCI Europe), cefoxitin sodium (EDQM, Strasbourg, France), cefpodoxime proxetil (EDQM), cefuroxime sodium (Sigma-Aldrich Chemie), efavirenz (TCI Europe), glibenclamide (ABCR, Karlsruhe, Germany), molsidomine (Sanofi-Aventis Deutschland, Frankfurt am Main, Germany), neostigmine bromide (Sigma-Aldrich Chemie), nitrofurantoin (Alfa Aesar, Heysham, United Kingdom), pyridostigmine bromide (Sigma-Aldrich Chemie), urea (Grüssing, Filsum, Germany), barbital (Sigma-Aldrich Chemie), barbital sodium (purchaser unknown), phenobarbital (Fagron, Barsbüttel, Germany), phenobarbital sodium (Synopharm, Barsbüttel, Germany), phenytoin (Sigma-Aldrich Chemie), phenytoin sodium (Sigma-Aldrich Chemie), prednicarbate (EDQM), tolbutamide (Sigma-Aldrich Chemie), zolmitriptan (TCI Europe).
Barium hydroxide solution *R* (47.3 g $Ba(OH)_2 \cdot 8\ H_2O$/L) was prepared according to Ph. Eur.

### *4.2. Equipment*

Centrifuge: EBA 8S (Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany).

### *4.3. Test reaction*

#### *4.3.1. Determination of the solubility of barium hydroxide in ethanol-water mixtures*

In order to find a suitable solvent for drugs with poor to moderate solubility in water, different ethanol-water mixtures were tested for their compatibility with barium hydroxide solution *R* (Ph. Eur.). The results are summarized in Table 2.

#### *4.3.2. Classification of investigated drugs on basis of their solubility*

Drugs with documented good water solubility were classified as „type A", indicating that the tests could be performed in aqueous solution without the risk of formation

**Table 2: Compatibility of ethanol-water mixtures with barium hydroxide solution *R***

| **Solvent** | **Addition of 1 mL barium hydroxide solution *R*** |
|---|---|
| 2 mL water[a] | no precipitate |
| 1.5 mL water + 0.5 mL ethanol[b] | no precipitate |
| 1 mL water + 1 mL ethanol | no precipitate |
| 0.5 mL water + 1.5 mL ethanol | no precipitate |
| 2 mL ethanol | precipitate |

[a] carbon dioxide-free water *R* was used; [b] anhydrous ethanol *R* was used.

of a precipitate upon addition of the barium hydroxide solution. All other drugs were treated with a mixture of 1 mL carbon dioxide-free water *R* and 1 mL anhydrous ethanol *R*. In case a clear solution was obtained, further 1 mL carbon dioxide-free water *R* was added. If the solution stayed clear after this addition, the substance was classified as “type B” drug. Compounds that did not dissolve in the ethanol-water mixture, or if a precipitate was formed upon addition of 1 mL water, were referred to as “type C” drugs and were not further investigated.

### *4.3.3. Tests*

Test solutions: For “type A” drugs: Dissolve 100 mg of the drug in 2.0 mL carbon dioxide-free water *R*. For “type B” drugs: Dissolve 100 mg of the drug in 1.0 mL carbon dioxide-free water *R* and 1.0 mL anhydrous ethanol *R*.

Method A (identification test C of trimethadione (Ph. Eur.)): To the test solution add 1 mL of barium hydroxide solution *R*. A white precipitate is formed, which dissolves on addition of 1 mL of dilute hydrochloric acid *R*.

Method B: To the test solution add 1 mL of barium hydroxide solution *R* and place the test tube on a water bath of 80 °C for 5 min. A white precipitate is formed. After cooling to room temperature the white precipitate dissolves on addition of 1 mL of dilute hydrochloric acid *R*. In the case of molsidomine the test was carried out on half scale.

Method C: In the case the mixture turned colored upon heating with barium hydroxide solution (cefoxitin sodium, cefuroxime sodium) or if precipitation of the drug or hydrolysis products was to be expected upon addition of hydrochloric acid (phenobarbital sodium, phenytoin sodium), the following modification was performed: To the test solution add 1 mL of barium hydroxide solution *R* and place the test tube on a water bath of 80 °C for 5 min. After cooling to room temperature centrifuge the mixture (2000 rpm, 5 min), separate the white precipitate, and wash it with 0.5 mL of water *R*. The precipitate dissolves on addition of 1 mL of dilute hydrochloric acid *R*. In the case of cefoxitin sodium this test was carried out on half scale.

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Conflicts of interest: none declared.

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