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The detection of doping by means of chromatographic methods

Manfred Donike*

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Doping has become an issue in elite sport and necessitates sensitive detection assays that enable the identification of organic compounds on a microscale level in urine. In agreement with modern toxicological methods, sports drug testing approaches can utilize paper, thin layer or gas chromatographic methods to reveal the presence of prohibited substances such as strychnine, pervitine, captagone, benzedrine etc. in doping control specimens. Basic principles of these strategies are summarized and considerations for future applications discussed.

Keywords: doping; sport; stimulants; narcotics

Doping is prohibited according to the regulations of most sporting federations and punished with sanctions; however, along with ethical considerations, these are apparently not sufficient to reduce or eliminate the misuse of drugs in sports. The only efficient way to combat doping seems to be the rigorous application of controls for competing athletes.

Based on the pharmacology of performance enhancing compounds used in sports, the detection of doped athletes is linked to the identification of organic substances on a microscale level. Excellent means for this task are provided by chromatography; paper,[1] thin layer,[2,3] and gas chromatography[4–6] have been successfully applied in modern toxicological assays. The practical aspects, and the theoretical backgrounds, are not be described herein; for further reading, the investigator is referred to additional monographic publications[7–10] and review articles.[1,2,4]

In the case of thin layer and paper chromatography, the Rf-value (Figure 1), which is defined as the ratio of the migration distance of the substance and the solvent, serves for the characterization of a compound. The Rf-value is a physical constant, comparable to boiling point, melting point, or refraction index. This, of course, applies only under compliance with constant conditions. Since Rf-values show considerable differences to the theoretical value with, for example, minute deviations in temperature, it is recommended to compare the investigated samples to authentic reference material.

An overview of separation possibilities is given in Tables 1 and 2.

Compounds of closely related chemical structure are separated as demonstrated, for instance, with Veritol–Ephedrine, Benzedrine–Pervitine, and Ephedrine–Pervitine. The usually colourless substances can commonly be localized on respective chromatograms by UV-light exposure; furthermore, colour reactions with sprayed reagents not only serve the purpose of visualization but also of characterization of the analytes. Employing inorganic supports during the manufacturing process of the thin layer chromatography plate, aggressive reagents such as chromosulfuric acid are applicable; this, in conjunction with a rapid developing time, commonly less than one hour, is considered an advantage.

For compounds that do not undergo decomposition during vaporization, gas chromatography yields excellent results. The separation of Amphetamine (Bp_{760} = 203–204 °C) and Pervitine (Bp_{760} = 208–210 °C) is given as an example in Figure 2.

The time between sample injection and registration at the detector is referred to as retention time. It is characteristic for a particular compound if constant conditions are maintained. For illustration purposes, a schematic drawing of a gas chromatograph is shown in Figure 3. Although appearing rather simple, maintaining constant conditions necessitates a considerable instrumental effort in practice.

Following this brief overview of chromatographic options for doping controls, the question of specimens arises. What kind of material could be obtained in the course of conducting doping tests? The best option is without doubt the collection of bodily fluids immediately after competition. Blood, saliva, sweat, and urine are eligible. Here, the urine sample will probably be the best choice to yield an unambiguous result as the concentration of the administered drug or its metabolites is the highest. In sports disciplines in which the athletes consume food and liquids due to lengthy competitions, nutrition can also be tested for doping agents. A search of luggage or clothing might also uncover pharmaceuticals. It is, however, not guaranteed that the labels on bottles discovered correspond to the actual content. Here also, a chemical analysis is required.

If there are no indicators of the use of a particular drug, the sample has to undergo a systematic toxicological analysis. Various authors have developed separation methods related to the Stas-Otto-process,[1,2,4] which enable a rapid and safe approach for the analysis of numerous pharmaceuticals employing chromatographic procedures. These and other methods reported in the

Manfred Donike, Institute of Organic Chemistry, University of Cologne, Zülpicher Straße 47, Cologne, Germany

Institute of Organic Chemistry, University of Cologne, Zülpicher Straße 47, Cologne, Germany

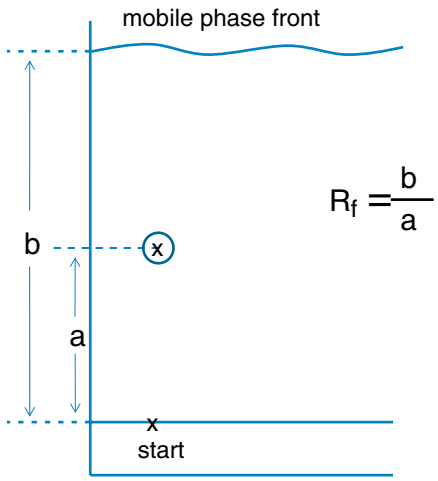

Figure 1. Determination of the Rf-value of doping agents with a = migration distance of the analyte and b = migration distance of the solvent.

Table 1. Paper chromatographic separation of selected pharmaceuticals (adapted from Vidic and Schütte[1])

a) Substance	Rf-Value*	b) Substance	Rf-Value**
Sympatol	0.0	Coramine	0.35
Veritol	0.07	Effortil	0.43
Ephedrine	0.22	Strychnine	0.44
Benzedrine	0.29	Ephedrine	0.54
Strychnine	0.57	Pervitine	0.62
Coramine	0.77	Cardiazol	0.81

Table 2. Thin layer chromatographic separation of selected pharmaceuticals.[3]

Substance	Rf-Value***
Benzedrine	0.16
Pervitine	0.09
Preludin	0.26
Ritaline	0.70
Katovit	0.78
Regenon	0.90
Captagone	0.54
Tradone	0.60

* mobile phase: dichloroethane, acetic acid, water (20 : 8 : 2, *v* : *v* : *v*)
** mobile phase: butanol, formic acid, water (12 : 1 : 7, *v* : *v* : *v*)
*** mobile phase: dimethylformamide, ethyl acetate (1 : 9, *v* : *v*) + 3 drops of n-octanol.

literature have a common element with using a pre-concentration and purification step of the aqueous sample, consisting of a liquid-liquid extraction at neutral, alkaline, and acidic pH by means of organic solvents. Chloroform, ether, cyclohexane, and other solvents are recommended. After removing most of the organic layer in a water bath, the remaining concentrated solutions are subjected to chromatography. The residue is applied to the start of a paper or thin layer chromatogram or undergoes

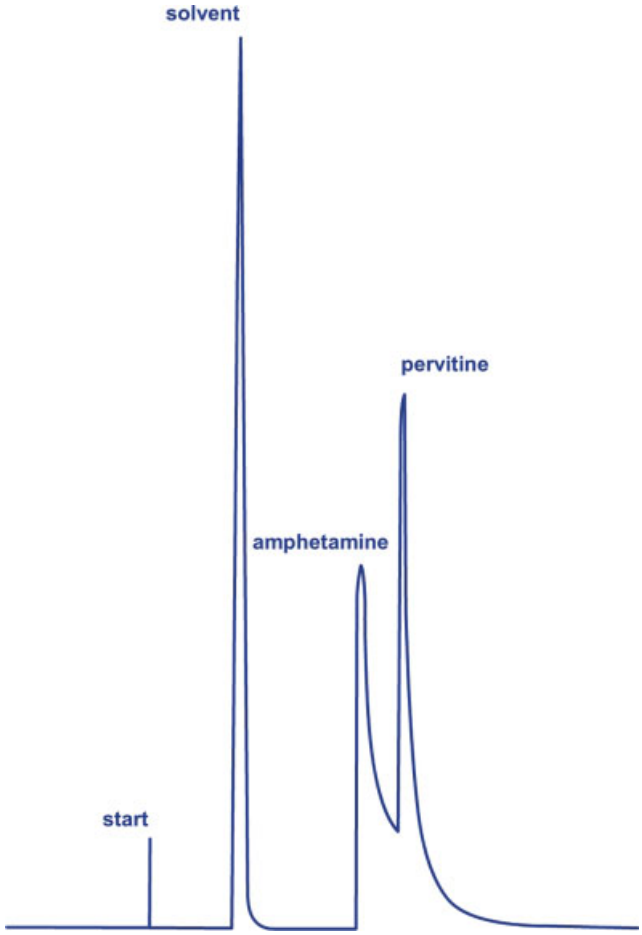

Figure 2. Separation of Amphetamine – Pervitine, capillary column, 100 m, Apiezon, column temperature 140 °C, carrier gas: helium (25 mL).

gas chromatography. A great number of toxicologically relevant substances are identified using this approach. Comprehensive tables are presented by Vidic,[1] Machata,[2] and Kazyak.[4]

In some cases, qualitative detection might not be sufficient. In such cases, the non-physiological administration of vitamins and hormones should be considered. The same applies to caffeine, for example, in drinks. Since such compounds are ingredients of nutrients and metabolism, respectively, a quantitative determination might be obligatory in contrast to the qualitative identification. All of the above-cited chromatographic methods allow for quantitative evaluation. For instance, using paper-chromatographic separation followed by visualization of the target substance, the total amount can be estimated from the spot size. Another frequently employed approach is briefly presented: labelling of the target compound under UV-light, elution with an appropriate solvent, and measurement of the extinction at an adequate wavelength. With the validity of Lambert's law ($E = \varepsilon \times c \times s$), the concentration c is calculated if ε is known. This method is particularly advantageous after thin layer chromatographic separation using inorganic supports as no interfering substances are liberated by the elution step.

In the course of data evaluation, the question of potential sources of error occurs.These can be eliminated by co-chromatography of authentic reference material and, in the case of paper or thin layer chromatography, by means of different mobile phases as well as spray reagents. Accordingly, columns of different

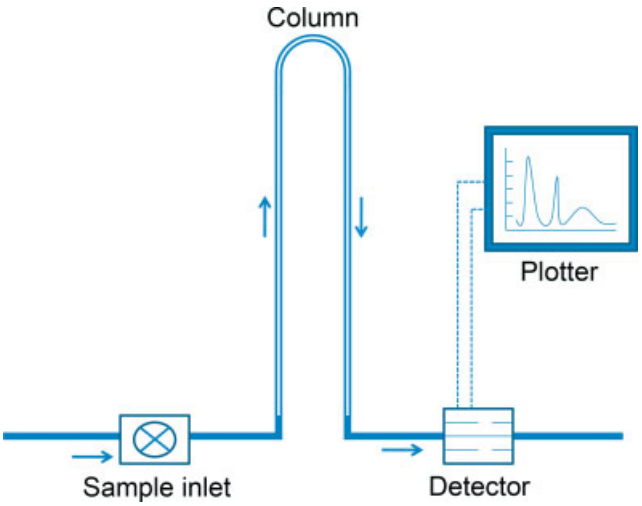

Figure 3. Schematic drawing of the setup of a gas chromatograph.

polarity should be used when gas chromatography is employed. If the chromatographic behaviour of a substance is identical to that of an authentic sample under these varying conditions, evidence for the identity of both compounds is provided. Moreover, the value of an analytical result concerning an isolated substance can further be corroborated using UV- or IR-spectroscopy. Another option that has been frequently employed is the combination of gas chromatography and thin layer chromatography. Here, the mixture of compounds is separated by gas chromatography. The individual substances are adsorbed on a thin layer chromatography plate directly from the gas exit valve and chromatographed in comparison to reference material.

For future purposes, the combination of gas chromatography and mass spectrometry appears to be the method of choice; both methods are applicable to compounds of high volatility. Besides retention time, the molecular mass of the analyte is obtained with highest accuracy.

Finally, a few words about the sensitivity of the chromatographic methods, exemplified using Amphetamine. Moerman[11] reports a detection limit for the thin layer chromatographic separation of 15–20 µg, Eberhardt and Debackere[3] of 3–10 µg depending on the employed spray reagent. In the case of paper chromatography, the limit of detection is commonly slightly higher than expected at around 30–40 µg. Gas chromatography allows the identification of 1 µg or less according to Cartoni and Stefano[12] as well as Kolb and Patt.[6] Particularly the amounts of stimulants detectable by means of gas chromatographic procedures are considerably lower than those expected in urine according to excretion rates reported in administration studies by Axelrod[13] and Alles and Wisegarver.[14]

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