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Radioimmunoassay of drugs of abuse in hair. Part 1: Methadone in human hair, method adaptation and the evaluation of decontamination procedures

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Abstract: A method suitable for the determination of methadone in human hair is presented. Adaptation and evaluation of a solid-phase I^{125} radioimmunoassay, designed for the quantitative measurement of methadone in urine, and development of a pre-analytical wash procedure has enabled a specific, sensitive and accurate analytical procedure to be developed. The specificity of the antiserum towards other drugs or biologically active compounds is evaluated up to a concentration of 100,000 ng ml^{-1} and accuracy covering a range of 0–450 ng ml^{-1} is found to be within 6% of expected methadone concentrations prepared in both drug free hair extract and urine. Inter-assay relative standard deviation (RSD) at concentrations of 5.1, 76.0 and 247 ng ml^{-1} methadone are 5.5, 2.5 and 3.6% respectively (n = 10) and intra-assay RSD at concentrations 2.3, 25.2 and 217 ng ml^{-1} are 5.3, 3.6 and 6.8% (n = 5). The limit of detection is 0.5 ng ml^{-1}. Extraction of control drug free hair samples spiked with methadone at concentrations of 100, 250 and 400 ng ml^{-1} achieved recoveries of 86, 80 and 89%, respectively. Control hair samples contaminated with methadone are examined under differing wash procedures to assess their effectiveness in the removal of methadone contaminant. A suitable pre-analytical wash regime is proposed for removal of contaminant derived from external or environmental sources. The mechanics of the wash action and contaminant application to the hair is discussed. It is concluded that the adapted radioimmunoassay and developed pre-analytical decontamination procedure is a suitable technique to employ for the measurement of methadone in human hair, be it prescribed or abused, with concentrations expressed as ng methadone per mg hair.

Keywords: *Hair; methadone content; radioimmunoassay; method adaptation; decontamination procedures.*

Introduction

Methadone, (±) -6-dimethylamino-4,4-diphenylheptan-3-one, is a synthetic narcotic analgesic used in the treatment of heroin and morphine addiction. In the United Kingdom it is used in the form of an oral linctus for withdrawal and maintenance programmes. By using methadone in this form, the risks associated with injecting are avoided; furthermore, the linctus has little value on the black market [1]. Although the action of methadone is similar to morphine, it is more difficult to metabolize. Thus it is more effective as an oral dose producing a long lasting action [2]. The principal metabolites found in urine are 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidone (EDDP) and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (EMDP) [3]. Methadone is available in tablet and injectable forms and has been subject to abuse giving rise to dependence in its own right [2].

The clinical laboratory plays an important role in the diagnosis and management of drug abuse and dependence by providing an independent and objective source of information. Urine, the routine specimen of choice, has established methodologies which are readily adapted to automation [4]. However, guidelines for collection to avoid adulteration and substitution need to be followed [5]. For urine samples the window of detection indicates recent drug usage. Typically, methadone and its metabolites can be detected for only 1–3 days post ingestion [6].

The last decade has seen an increased interest in the potential use of hair analysis for the detection of drug usage. Scalp hair in the posterior vertex region grows at a relatively constant rate of 1 cm per month [7]. Analysis of human hair, therefore, provides a means of determining long-term drug abuse histories as traces of drugs and their metabolites are laid down in hair during keratinization and remain

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embedded throughout its life [8], the length of the hair sampled being the limiting factor. Furthermore, the development of a segmental form of analysis has enabled the pattern of drug usage to be studied on a month by month basis [9, 10].

The absolute mechanism of drug incorporation into the hair is little understood, much of the information being gained from research in the cosmetics industry [11].

Hair may be contaminated with drugs as a result of environmental exposure or through adsorption onto the hair from perspiration, sebum or drug handling. This issue is particularly important when drugs such as cocaine, heroin, phencyclidine and cannabis are smoked [12]. Drugs are not normally absorbed through the cuticle into the cortex layer of the hair although the far distal end of the shaft shows progressive loss of cuticle, so perhaps should be rejected from an analytical standpoint [10].

Drugs of abuse are controlled substances and it is unlikely that intentional or accidental application to the hair would occur. External contamination on the outer layers of the hair is best removed by inclusion of a wash step prior to analysis [13], or if necessary can be distinguished from blood derived drugs by analysis of the washes or detection of metabolites [14].

Recent years have seen the publication of work concerning the detection of opiates [8, 9, 15–17], amphetamines [18, 19] and especially cocaine [20–25] in hair with respect to drug abuse. However, there have been very few published works on the detection of methadone in human hair [26, 27].

The authors have developed a procedure for the measurement of methadone in human hair by adapting a solid phase radioimmunoassay, (Coat-a-Count, DPC Los Angeles, CA), designed for the quantitative measurement of methadone in urine. Controls have been included to evaluate the ability of wash procedures in eliminating heavy 'environmental' contamination prior to analysis.

Experimental

Reagents and materials

Methadone hydrochloride was obtained from Sigma Chemicals (Poole, Dorset). Analar grade acetone and methanol, dodecyl sulphate, hydrochloric acid and sodium hydroxide were obtained from Merck Ltd (Poole, UK). Phosphate buffer (Sørensen) [28] was prepared from Merck reagents. Analysis for hair methadone was by solid-phase radioimmunoassay, (RIA), using a Coat-a-Count system (DPC, Los Angeles, CA, USA). The method for the determination of methadone in urine has been adapted in this work to enable the quantitative analysis of hair. The claimed high specificity for methadone and extremely low cross reactivity to other drugs or compounds was confirmed by the analysis of a range of abused drugs and prescribed substances.

Apparatus

An LKB-Wallac 1260 Multigamma II counter controlled by a RIA Calc program, operated on a Hermes PC 110 (Pharmacia LKB Biotechnology, Milton Keynes, UK) and linked on-line to an Olivetti DM282 printer, was used to simultaneously count 12 samples of a gamma emitting isotope. The assay tubes are instrument compatible and its protocol is installed via the computer.

Oxford 800 series mono and repetitive syringe pipettes (Boehringer Mannheim, Mannheim, G) were used for the addition of sample/calibrators and [I^{125}] methadone respectively. Mixing was carried out using a vortex mixer and weighing procedures performed on a Gallenkamp Mettler H20 balance (Fisons Instruments, Loughborough, UK).

Assay adaptation

The Coat-a-Count system was evaluated in terms of specificity, accuracy and both inter- and intra-assay precision. A standard curve was prepared from stock methadone hydrochloride dissolved in hair extract from known drug free samples. The stock solution was diluted to various concentrations in drug free hair extract for comparison with the urine calibrators supplied with the kit. Drug free hair samples were spiked with known quantities of methadone hydrochloride prior to extraction for quality control and recovery purposes in all analytical processes using the Coat-a-Count system.

Hair sample collection

Full length hair samples were obtained from known drug free volunteers by cutting the hair close to the scalp and tying the cut end together with cotton thread. Samples were stored in a drug free environment.

Preparation of contaminated hair samples

Each sample was divided into full length sub-samples, of approximately equivalent weight, and used in one of the following contamination models. (In-house trials have shown that for ease of handling, the minimum sample size of hair should be greater than 10 mg).

Models A and B. The prewashed drug free hair samples were contaminated with methadone by either overnight soaking in a solution of 0.1 mg ml^{-1} methadone at room temperature, model A, or by rubbing a solution of 0.1 mg ml^{-1} methadone along the outer layer of the hair until it appeared wet, model B. The contaminated samples were removed to a drug free environment and allowed to dry naturally in air.

Models C and D. A prewashed drug free hair sample, cut at each end, was divided into two lengthwise and each further subdivided into four sub-samples. One set of samples remained unchanged whilst the other had the cut ends sealed with paraffin wax. Both sealed and unsealed samples were contaminated by overnight soaking, at room temperature, in solutions of 0.1 mg ml^{-1} methadone, model C, or 0.05 ng ml^{-1} methadone, model D. The contaminated samples were removed to a drug free environment and allowed to dry naturally in air.

Each contamination model contained samples identically treated with distilled water in place of contaminant, for the provision of sample blanks.

Hair wash procedures

The following wash routines were applied to the contaminated hair samples:

Model A. Three wash protocols were used, namely:

(i) methanol at 37°C for 15 min, 2.5 ml each × 4;
(ii) acetone at room temperature for 15 min, 2.5 ml each × 2 followed by distilled water 2.5 ml each × 2;
(iii) dodecyl sulphate (1% w/v aqueous) at 37°C for 15 min, 2.5 ml each × 3 followed by distilled water 2.5 ml each × 2.

Model B. Two wash protocols were used, namely:

(i) methanol at 37°C for 15 min, 2.5 ml each × 4 followed by distilled water 2.5 ml each × 2.
(ii) dodecyl sulphate (1% w/v aqueous) at 37°C for 15 min, 2.5 ml each × 4 followed by distilled water 2.5 ml each × 2.

Model C. A single wash with dodecyl sulphate (1% w/v aqueous) at 37°C for 15 min, 2.5 ml each × 4 followed by distilled water 2.5 ml each × 2 was used.

Model D. A single wash with dodecyl sulphate as described in Model C.

A proportion of the contaminated hair samples was not subjected to the routines for comparison purposes. All samples were left to air dry, the dry weight of each hair sample being determined prior to extraction. The waxed ends of the samples so treated were removed before weighing and extraction.

Extraction and analysis

Hair samples contained in 3.5 ml Röhren-Tubes, (Sarstedt, Newton, NC, USA) were immersed in 1 ml of 0.1 M hydrochloric acid and incubated overnight at 55°C. Each extract, 0.5 ml, was neutralized with 0.05 ml of 1.0 M sodium hydroxide, and finally diluted with 0.45 ml of 0.1 M phosphate buffer, pH 7.0. The buffered extracts were analysed for methadone concentration by RIA. Twenty five microlitres of calibrators, controls and hair sample extracts were incubated at room temperature for 1 h in antibody coated tubes with 1 ml of I^{125} labelled methadone tracer. The thoroughly decanted tubes were counted on the gamma counter. The measured radioactivity, as counts per minute was converted to the equivalent ng ml^{-1} with reference to the computed calibration curve, which covered the range from 0 to 500 ng ml^{-1}. Sample concentrations above this range were diluted, as appropriate, and re-analysed.

The serial washings from protocol Model A were individually collected and analysed for methadone to determine how effective the wash protocols had been in removing methadone from the contaminated hair samples. The final wash only was collected from protocols B, C and D for analysis as deemed necessary. The levels of methadone determined in the unwashed, washed and washings were compared.

Results and Discussion

The methadone concentrations were calculated using a logit-log format incorporating a smooth spline fitting algorithm. Drug concentrations in hair samples were expressed as ng methadone per mg hair.

Assay adaptation

The antiserum specificity was examined up to a level of 100,000 ng ml^{-1}, covering a range of abused substances and prescribed drugs. The observed data were compared with the manufacturers specifications to verify the claimed high specificity for methadone (see Table 1). Accuracy over the range 0–400 ng ml^{-1} was found to be within 6% of actual concentration. Comparison between supplied methadone standards prepared in drug free human urine and in-house methadone standards prepared in drug free hair extract also were within 6% of expected values, over the range 0–450 ng ml^{-1}. On this evidence the analysis of methadone as measured by the Coat-a-Count system appears to be applicable to the determination of methadone in hair extracts. Previous experimental data on a population of known non-drug abusers, (n = 19), has indicated that levels below 0.15 ng methadone per mg hair should be considered negative [unpublished results]. The population contained both male and female volunteers, age range 16–40 years (mean 29 years) and hair samples of varying colour and ethnic origin. The above level is similar to findings of other workers [26].

The inter-assay RSD at mean methadone concentrations of 5.1, 76.0 and 247 ng ml^{-1} were 5.5, 2.5 and 3.6%, respectively, with corresponding SD of 0.28, 1.86 and 8.9 (n = 10). Intra-assay RSD at mean methadone concentrations of 2.3, 25.2 and 217 ng ml^{-1} were 5.3, 3.6 and 6.8%, respectively, with corresponding SD of 0.12, 0.91 and 14.8 (n = 5). The limit of determination of the methadone by RIA was found to be 0.5 ng ml^{-1}. The above results were comparable to stated performance characteristics of the Coat-a-Count System (Technical Information sheet G153, DPC, Los Angeles, CA, USA). Recovery of methadone added to washed control hair following acid extraction was 86, 80 and 89%, respectively, for the spiked concentrations of 100, 250 and 400 ng ml^{-1} methadone.

Decontamination evaluation

The results from the various decontaminating procedures as described in Models A, B, C and D are shown in Tables 2–5. Serial washes collected from one of the duplicates in Table 2

Table 1
Examination of specificity for methadone antiserum

Compound	Added concentration (ng ml^{-1})	% Cross reaction (observed)	% Cross reaction (claimed)
Morphine	100000	0.011	N.D.*
Phencyclidine	100000	0.018	N.D.
Dipipanone	100000	0.51	N.C.†
Oxazepam	100000	0.00004	N.D.
Amphetamine	100000	0.0012	N.D.
Phenobarbitone	100000	0.0004	N.D.
Cocaine	100000	0.0011	N.D.
Dihydrocodeine	100000	0.0028	0.003
Pholcodeine	100000	0.006	N.C.
Thioridazine	100000	0.084	N.C.
Haloperidol	100000	0.012	N.C.
Riboflavin	10000	0.17	N.C.
Quinine	10000	0.18	N.C.
Chlorpromazine	100000	0.063	0.03
Imipramine	100000	0.035	0.02
Diphenhydramine	10000	0.08	0.02
Propoxyphene	100000	0.03	0.02
Methadone	1000	99.6	100
EDDP (Methodone metabolite)	10000	0.013	0.01

*Non detectable. †Not checked by manufacturer.

Antiserum claims high specificity for methadone with low cross reactivity to other drugs or compounds that may be present in patient samples. The above compounds were obtained from commercial sources and solutions prepared in blank urine for assay up to a level of 100,000 ng ml^{-1}.

Table 2
Efficiency of wash procedures for removal of methadone from contaminated hair samples*

Wash method and model	Methadone concentration ng ml^{-1} hair Removed by wash	Acid extract fraction	% Methadone removed by wash
Methanol — Model A	1894	855	69
	2289	775	75
Dodecyl Sulphate — Model A	1699	1050	62
	2042	1022	67
Acetone — Model A	1066	1683	39
	1313	1751	43
Unwashed	None	2749	None
	None	3064	None

* Duplicate drug free samples analysed for each protocol.

Table 3
Efficiency of wash procedures for removal of methadone from contaminated hair samples*

Wash method and model	Methadone concentration ng ml^{-1} hair Removed by wash	Acid extract fraction	% Methadone removed by wash
Methanol — Model B	17.56	0.04	99.8
	15.47	0.13	99.2
Dodecyl Sulphate — Model B	17.27	0.33	98.1
	15.59	0.01	99.9
Unwashed	None	17.6	None
	None	15.6	None

* Duplicate drug free samples analysed for each protocol.

Table 4
Efficiency of wash procedures for removal of methadone from contaminated hair samples*

Wash method and model	Methadone concentration ng ml^{-1} hair Removed by wash	Acid extract fraction	% Methadone removed by wash
Dodecyl Sulphate —	380.7	50.3	88
Model C — Unsealed	380.8	50.2	88
	377.2	53.8	87
Unwashed-Unsealed	None	431	None
Dodecyl Sulphate —	269.9	18.1	94
Model C — Sealed	266.3	21.7	93
	273.9	14.1	95
Unwashed-Sealed	None	288	None

* Triplicate drug free samples analysed for each protocol.

Table 5
Efficiency of wash procedures for removal of methadone from contaminated hair samples*

Wash method and model	Methadone concentration ng ml^{-1} hair Removed by wash	Acid extract fraction	% Methadone removed by wash
Dodecyl Sulphate —	416.7	31.3	93
Model D — Unsealed	427.5	20.5	95
	413.4	34.6	92
Unwashed-Unsealed	None	448	None
Dodecyl Sulphate —	791.6	23.4	97
Model D — Sealed	798	17	98
	803.7	11.3	99
Unwashed-Sealed	None	815	None

* Triplicate drug free samples analysed for each protocol.

were analysed to show the effect of successive washings in the removal of the methadone contaminant; the results are summarized in Fig. 1. Removal of methadone contaminant from drug free hair by methanol, acetone and aqueous dodecyl sulphate, as detailed in Model A, showed the mean effectiveness to be 72, 41 and 65%, respectively, at a contaminant level of 0.1 mg ml^{-1} methadone. Analysis of the serial washes collected showed that the majority of contaminant was removed during the first wash in each case. However, the washing procedures of Model A were not sufficiently effective in removing methadone contamination at the 0.1 mg ml^{-1} level. Acid extraction of the washed hair samples revealed remaining levels of 815 ± 40, 1717 ± 34 and 1036 ± 14 ng methadone per mg hair, following wash procedures with methanol, acetone and aqueous dodecyl sulphate, respectively.

During the process of overnight soaking the methadone solution, used as contaminant, would have access to the internal hair canal via the cut hair ends. It is possible that this internal

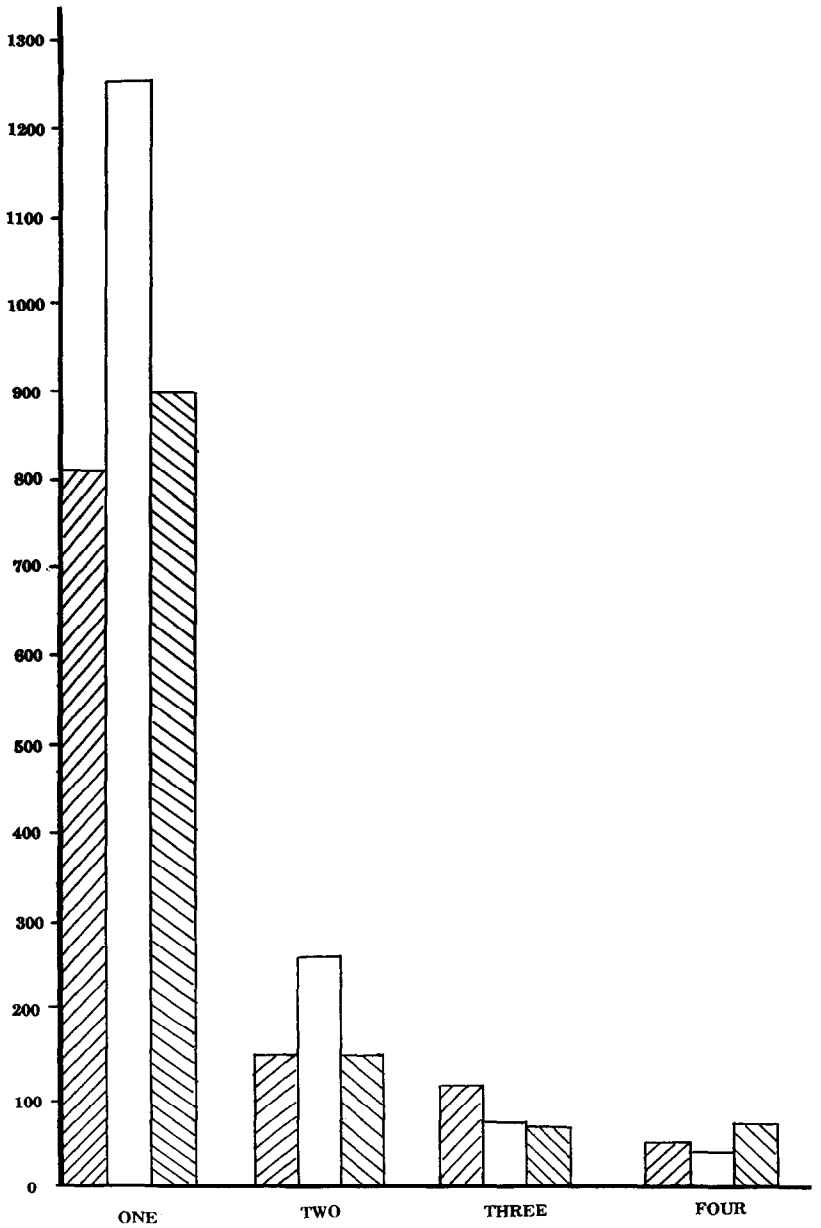

Figure 1
Concentration of methadone found in successive washings collected from contaminated hair samples following wash routines with dodecyl sulphate, methanol and acetone, as described for Model A. Plot of ng methadone/mg hair versus number of washes, one, two, three, four. ▨ Aqueous dodecyl sulphate. □ Methanol. ▧ Acetone.

contamination is more difficult to remove than contamination on the outside of the cuticle layer of the hair, as would be encountered in true forms of environmental contamination associated with drug taking habits.

In Model B the contaminant, 0.1 mg ml^{-1} methadone was applied to the outer layer of the hair only. The acetone wash protocol was omitted due to its apparent ineffectiveness. The prepared contaminanted hair samples were washed as detailed in Model B, the number of sequential washings being increased to aid the effectiveness. Both wash protocols were found to be greater than 98% effective in removing the methadone contaminant from all samples. Acid extractions, post washing, were found to contain levels of less than 0.35 ng methadone per mg hair in all cases. Therefore, the wash protocols used would appear to be efficient in the removal of the methadone contaminant, at the 0.1 mg ml^{-1} level, as applied to the outer layer of the hair.

The hair samples used for the Model C wash procedure were taken from a single drug free subject so a comparison of the contamination processes could be made on an individual sample.

During the overnight soaking the methadone solution would have access to the cut ends of the unsealed samples as well as the external layer of the hair. In the case of the sealed samples, access would mainly be via the external layer of the hair, although total sealing of the cut ends could not be fully guaranteed.

The levels of methadone contaminant detected from the extract of the unwashed and unsealed hair sample was 33% higher than that from the unwashed sealed sample. Considering the original sample was taken from the head of one individual it would appear that the sealing process has had an affect on the retention of methadone contaminant by the hair. As the only difference between the samples was access or not to the cut ends of the hair, it may be postulated that this increased retention is due to the methadone solution entering, by capillary action, the central core via the cut ends.

The wash protocol was more effective in removing methadone contaminant from the sealed hair samples, mean 94 ± 1%, than from the unsealed samples, mean 87.5 ± 0.5%. This suggests it is harder to remove the contaminant from the unsealed samples. The fact that the recovery of methadone from the sealed samples was incomplete suggests that some access to the central core via cut ends may have occurred due to inefficient sealing. When these results are compared to those obtained in experimental Model B, where the contaminant was applied to the outside of the hair only, this assumption gains support. Analysis of the final washing collected from both sealed and unsealed samples was found to be less than 0.3 ng methadone per mg hair in all cases. This adds further support to the theory that the contaminant can access via the cut ends of the hair sample into the central canal, from which it is more difficult to remove the contaminant than from the external surface of the hair. However, acid extraction of the hair enables the recovery of methadone from both the external and internal surfaces.

The results obtained for contamination procedure Model D support the findings of Model C. The previously described wash protocol also was found to be more effective in removing the methadone contaminant, 0.05 mg ml^{-1}, from the sealed hair samples than from the unsealed hair samples. However, in this experimental model the contaminant removal appeared to be more successful, mean 98 ± 1% for sealed hair samples and 93% for unsealed hair samples, respectively. Although the concentration of methadone contaminant used was less than in Model C, the thesis that the contaminant can enter the central hair canal via the open cut ends is still supported. Determination of the methadone concentration in the collected final washes was found to be less than 0.2 ng methadone per mg hair in all cases.

It may be concluded that the adapted RIA method is suitable for the measurement of methadone in human hair and the extended aqueous dodecyl sulphate wash protocol appears to be effective in the removal of methadone contaminant, applied to the external surface of the hair, up to a level of 0.1 mg ml^{-1}. Confirmation of the success of the decontamination procedure is obtained by analysis of the final wash solution.

Approaches using enzyme digestion for extracion of drugs has been used [14, 21]. Such conditions for total digestion of the hair may destroy the analytes of interest and under optimum conditions may react with antibodies in the RIA causing their destruction, particularly if any excess active enzyme remains. The employment of milder conditions for enzyme

digestion may lead to differing degrees of dissolution and irreproducible results [29].

Research on the analysis of morphine in human hair has shown presentation of the drug above the scalp some 7–8 days post ingestion [15]. There is concern regarding the possible effect of hair treatments on the analysis of drugs in hair. Studies have found that some drug levels can be reduced but probably not to the point of elimination [8, 29]. However, it is possible to use hair samples from other areas of the human body should the need arise.

The above results suggest that the adapted RIA combined with a pre-analytical decontamination procedure is suitable for the measurement of methadone in human hair. The method can be used to detect methadone be it prescribed or abused. Further work in which the analytical routine is applied to hair samples from known drug abusers is in progress and the results will be published in a forthcoming communication.

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