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Radioimmunoassay of drugs of abuse in hair Part 2: The determination of methadone in the hair of known drug users

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Abstract

This communication addresses the analytical problems associated with the analysis of hair specimens from known users and misusers of the synthetic opioid methadone. An adapted radioimmunoassay and a previously developed preanalytical decontamination procedure have been applied to samples from known drug users. The removal of drugs from the hair surface by washing and the effect of proprietary hair treatments on methadone entrapped in the hair have also been investigated. Pre-analytical washing reduced methadone levels by up to 29%, whilst hair colouration and peroxide bleaching were found to reduce levels by up to 21% and 50% respectively. Methadone assay of extracts from dated hair segments were shown to provide long term histories of methadone intake, under controlled and non-controlled conditions. Evidence that a dose relationship between hair drug levels and intake may exist is presented. Results of hair analysis, expressed as ng methadone/mg hair, from drug users (range 0.20–10.63) are compared to a pre-determined cut-off of 0.1 ng methadone/mg hair, obtained from the analysis of a known drug free population ($n = 23$).

Keywords: Hair; Methadone content; Patient detoxification; Radioimmunoassay; Segmentation; Washing

1. Introduction

Testing for drug misuse by urine analysis is a well established procedure that is highly adaptable to automation [1]. Urine drug levels generally reflect drug abuse 1–3 days prior to sampling, and are influenced by dose levels and time since last intake. Typically, methadone can only be detected for up to 3 days following administration. It is necessary to ensure that established protocols for the collection of urine specimens for drug misuse are rigorously adhered to in order to prevent problems arising from specimen adulteration and/or substitution [2]; otherwise, urine analysis results can be meaningless. The determination of drugs of abuse by hair analysis provides the potential for examination over a much larger timescale. Hair is a keratinous protein, the roots of which, contained in the follicles, are supplied with nourishment through a network of surrounding blood vessels [3]. If present in the blood plasma, drugs and their metabolites become embedded in the hair structure during the process of keratinisation and remain there throughout the lifetime of the hair. To date, the mechanisms of incorporation into the hair are not fully understood. Nevertheless, it has been established that analysis of hair can

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provide useful information concerning the presence of drugs and their metabolites in body fluids at the time of a particular keratinisation event [4].

It is well known that areas of the body are covered by differing types of hair. Variations in colour, texture, growth pattern and rates of hair growth can be influenced by both race and sex [5]. This stresses the importance of an agreed site location from which to sample hair for subsequent drug analysis. Scalp hair in the posterior vertex region grows at a relatively constant rate of 1 cm per month and is less influenced by the above-mentioned factors [4]. The relatively constant growth rate at this site provides hair analysis with a detection window ranging from months to years, thereby giving a unique opportunity for retrospective investigation of drug abuse [6,7]. Compartmentalisation of the hair sample using segmental regimes allows insight into the pattern of the drug use of an individual throughout well defined periods, e.g. at monthly intervals [6,7].

Reported studies on South American mummies [8] and certain 19th century poets [6] has provided evidence of the long term stability of drugs in hair samples, which suggests that the storage, preservation and transportation problems associated with other biological samples, used for drug analysis, are not applicable to hair. The non-invasive sampling techniques employed may also preclude hazards associated with other biological specimens, such as HIV and Hepatitis B infections.

Methods for the determination of a range of drugs and their metabolites in hair including opiates [9,10], morphine [6,7,11–13], cocaine and its metabolite benzoylecgonine [11,13,14–16], amphetamine [17–19], and other illicit substances [20,21], have been reported. However, published work on the detection of methadone in hair appears to be limited [22,23].

The previous paper in this series presented work on the adaptation of a urine methadone procedure to the analysis of methadone in hair by radioimmunoassay and also the evaluation of decontamination procedures to remove drug material derived from external sources, prior to analysis [24]. The present communication addresses the analytical problems associated with the assay of hair specimens from known users and misusers of the sythetic opioid methadone. The developed adapted radioimmunoassay procedure has been used to investigate environmental exposure, effects of certain hair treatments, methadone levels in users' hair under controlled and non-controlled conditions, and the establishment of a cut-off level based on a population of known non-abusers of methadone.

2. Experimental

2.1. Apparatus

Reagent, sample and calibrator additions were carried out using Oxford 800 mono and repetitive syringe pipettes (Boehringer Mannheim, Mannheim, Germany). Mixing was achieved by a vortex technique and weighing procedures were carried out using a Gallenkamp Mettler H20 balance (Fisons Instruments, Loughborough, UK). Subsequent radioactive counting was performed on an LKB-Wallac 1260 Multigamma-2 counter controlled using a RIACalc programme operated by a Hermes PC110 (Pharmacia LKB Biotechnology, Finland). The multidetector had the capability of simultaneously counting 12 samples of a gamma-emitting isotope. Protocols for the assay were stored on the computer.

2.2. Reagents

Hydrochloric acid (0.1 M), sodium hydroxide (1.0 M), dodecyl sulphate, analar grade methanol and phosphate buffer reagents (Sørensen) [25] were obtained from Merck Ltd. (Poole, UK). Methadone hydrochloride was obtained from Sigma (Poole, UK). Cream peroxide bleach (12%) (Jerome Russel Ltd., UK) and cream hair colourant (Roebuck Brown, Koleston, Wella Ltd., UK) were obtained for the hair treatment procedures.

Hair methadone analysis was carried out by a solid-phase radioimmunoassay technique using a Coat-a-Count system (DPC, Los Angeles, CA, USA). This consisted of methadone antibody coated tubes, ^{125}I-labelled methadone and methadone calibrators in methadone-free human urine. This method uses a solid-phase antiserum highly specific for d/l methadone with minimal cross reactivity to other compounds or drugs that may be present in patient samples (Technical Information sheet G153, DPC, Los Angeles, CA, USA). The procedure, designed for the measurement of methadone in urine, has been adapted and validated for the analysis of methadone in human hair samples [24].

2.3. Hair specimens

Full length samples of hair were taken, with patient consent, from the posterior vertex region in each case. The aligned cut end was tied off with cotton, for future reference, before individual storage in a drug-free environment. Drug-free samples were taken from volunteers ($n = 23$). Hair samples from 19 methadone users and misusers were provided with consent by opiate addicts in treatment at the Maudsley/Bethlem Royal Hospital drug dependence in-patient treatment unit.

2.4. Hair sample washing

Hair samples from three known methadone users were divided into three full length sub-samples of approximately the same weight, and subjected to different wash protocols prior to extraction and analysis, as follows:

(i) unwashed;

(ii) four methanol washes (2.5 ml) each for 15 min followed by distilled water (2.5 ml × 2) for 15 min at 37 °C;

(iii) four dodecyl sulphate (1%) washes (2.5 ml) followed by distilled water (2.5 ml × 2) for 15 min at 37 °C.

Labelled samples were left to dry naturally in a drug-free environment before the strategies were assessed as to their ability to remove material derived from external sources, which could lead to the misinterpretation of results.

2.5. Hair treatments

Pre-washed hair samples from known methadone users were divided into three full length sub-samples of approximately the same weight. One was left untreated for control purposes, and the remaining two sub-samples were treated according to one of the following procedures:

(i) 30 min treatment with cream peroxide bleach (12%).

(ii) 40 min treatment with cream hair colourant.

The samples were rinsed in warm distilled water (≈37 °C) to remove excess bleach or colourant adhering to the external surface of the hair, which was then allowed to dry naturally.

The treated and untreated sub-samples were washed using the previously described dodecyl sulphate procedure, before being allowed to dry naturally, again in a drug-free environment. The methadone content of each hair sub-sample was determined by radioimmunoassay.

2.6. Hair segmentation

A previously developed segmental hair analysis regime [7] was used to monitor drug use in methadone users over a period of months. The measured segments, starting from the scalp end, were individually washed using the dodecyl sulphate protocol and left to dry naturally in a drug-free environment, prior to analysis. Prescribed medication and self-report histories of methadone use corresponding to the periods of segmentation, if available, were obtained for corroboration.

The segmental regime and preparation of hair segments was also used to study a patient undergoing detoxification with methadone in order to study methadone deposition during the detoxification procedure.

2.7. Hair methadone concentration and methadone intake

Hair samples, taken with consent from 19 admissions to the in-patient treatment unit, were analysed to investigate possible correlation between methadone intake and hair methadone concentration. The volunteer group had been taking prescribed methadone, as maintenance, for at least a month, plus other illicit drugs in some cases, prior to admission. Individual comparisons were made based upon prescribed methadone, self-reported methadone use and the concentration of methadone present in the hair segment corresponding to a period of 1 month prior to admission. The hair samples were washed individually and left to dry prior to analysis, as described previously.

2.8. Hair methadone extraction and analysis

The dry weight of each prepared hair sample was determined prior to extraction. The samples were immersed in 0.1 M hydrochloric acid and incubated overnight at 55 °C. Neutralisation with equimolar amounts of 1 M sodium hydroxide was followed by addition of 0.1 M phosphate buffer (pH 7) to the hair extract. Prepared extracts were analysed for methadone using solid-phase radioimmunoassay. Extracts from drug-free hair samples that had been

spiked with known concentrations of methadone were analysed for recovery and quality control purposes. All results were converted to the equivalent ng methadone/mg hair concentration and assessed accordingly.

3. Results and discussion

The results from duplicate sample measurements were calculated using a logit–log representation. The suitability of the Coat-a-Count system for measuring methadone in hair samples has been evaluated in detail previously [24]. Antiserum specificity up to levels of 100 000 ng ml^{-1} potential interferant has been examined and accuracy of the assay up to a concentration of 450 ng ml^{-1} verified. The performance of the adapted assay with respect to inter- and intra-assay RSD was found to be consistent with the manufacturer's specification.

Drug-free hair samples were spiked with known concentrations of methadone hydrochloride prior to extraction for quality control and recovery purposes in all analytical processes involving the Coat-a-Count system. Hair samples from known methadone users and non-drug users were employed as qualitative controls. The recovery of methadone added as spike to washed control hair following acid extraction was 100, 90, 79, 89 and 85% respectively for spiked concentrations of 0.0, 75, 100, 400 and 500 ng ml^{-1} methadone.

Assay of hair from a population of known non-drug users indicated that levels below 0.1 ng methadone/mg hair may be considered as negatives. This value was derived from a population of 23, with a mean age of 26 years (range 16–40 years) and comprised of both males and females from different ethnic groups and with varying hair colouration.

3.1. Hair washing in methadone users

Pre-analytical washing is a necessity in hair drug analysis for the removal of extraneous material derived from external sources. Failure to include a washing process can lead to false analytical results owing to the accidental contamination of hair samples. Methadone levels detected in the hair samples from three known methadone users, following wash protocols, are shown in Table 1. In each case a small but significant decrease in hair methadone levels was detected on the introduction of a pre-analytical wash protocol. The mean reduction in hair methadone concentrations following pre-analytical washing were 29%, 15% and 28%, respectively, for subjects A, B and C (Fig. 1). Washing would appear to remove loosely bound drug that may have been absorbed onto the outer surface of the hair via perspiration, sebum and possible drug handling. In addition, it is possible that some drug from the internal structure of the hair may have been removed if the hair was damaged either naturally or chemically. Indeed, it has been shown that up to 66% of total morphine can be removed by washing, prior to analysis, using strong solvents such as diethyl ether and dilute hydrochloric acid [26]. However, it is essential that a pre-analytical washing step is included, as the changes in methadone concentration for subjects A, B and C could influence conclusions concerning the historical record of drug usage of a patient and claims of abstinence [27].

Table 1
Methadone levels in hair samples of known users

Washing protocol	Concentration of methadone (ng per mg hair) [a]		
	Subject A	Subject B	Subject C
None	2.03	12.45	7.46
Methanol–water	1.30	10.68	5.36
Dodecyl sulphate–water	1.60	10.52	5.43

[a] Subject A, red hair; Subject B, dark brown hair; Subject C, light brown hair.

3.2. The effect of proprietary hair treatment upon the methadone contents of hair from known users

Two over-the-counter hair treatment products were investigated to determine the effect each had on the methadone content of hair from known users. The results obtained after treatment of the pre-washed hair with cream hair colourant and cream peroxide bleach (12%) are shown in Fig. 2. Although the hair samples were no longer attached to the scalp of the subjects, the treatment conditions were made as realistic as possible. The cream peroxide treatment had a marked effect upon the methadone content of the hair of the three subjects, with values being reduced by between

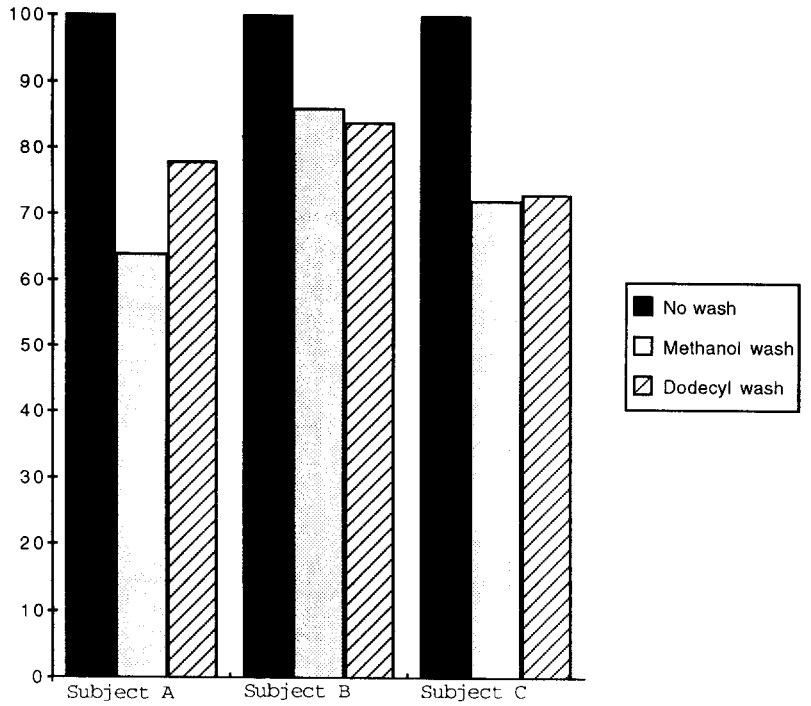


Fig. 1. Percentage methadone remaining in hair following pre-analytical washing procedures in known methadone users.

25 and 50% on direct comparison with the untreated sub-sample. The colourant treatment again resulted in a decrease, but less marked, being in the range 6–21%. In both instances, the losses could result from methadone leaching out from the internal structure of the hair owing to chemical damage or chemical modification of the analyte, causing a lack of recogni-

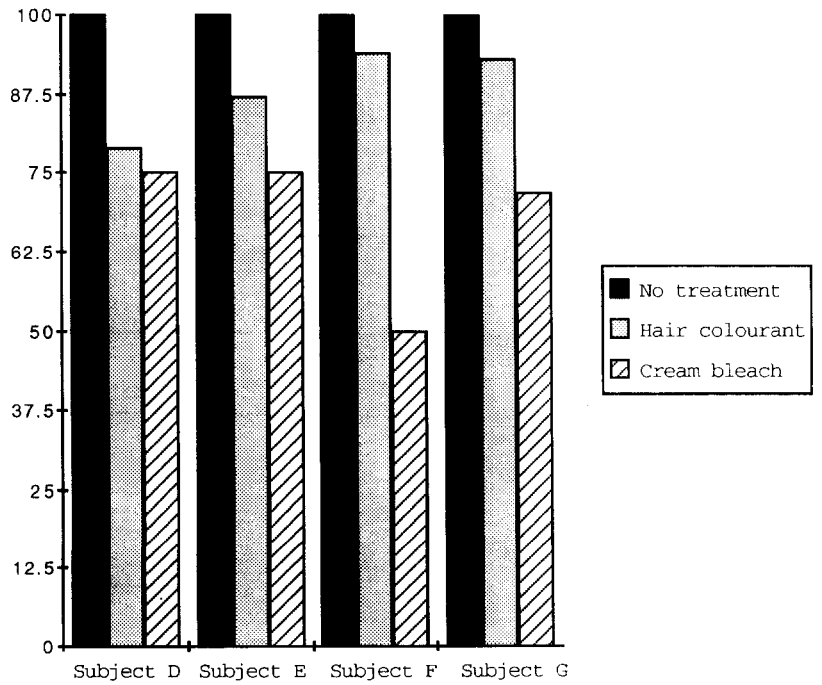
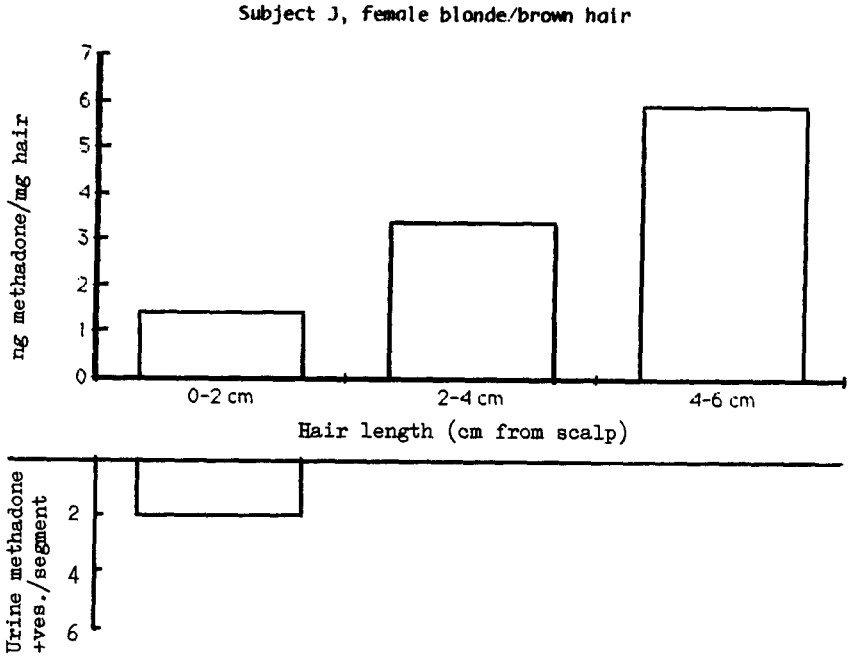


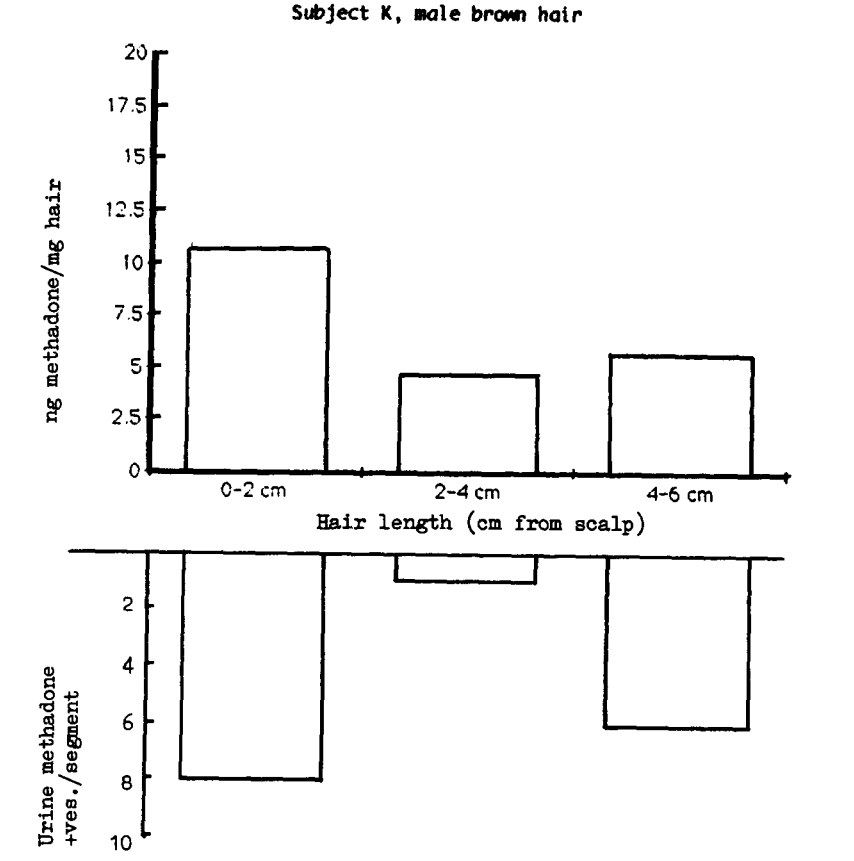
Fig. 2. Effect of hair treatments on samples from known methadone users. Subject D, female, dark brown hair; Subject E, male, brown hair; Subject F, female, auburn hair; Subject G, male, brown hair.

tion of the modified analyte by the methadone antibody used in the assay.

Although not tested, it is anticipated that habitual peroxide use could decrease hair methadone content even further. Consistent with this view, subject H, a peroxide blonde who continually urine tested positive for methadone over a period of a year, prior to hair sampling, gave a detectable methadone level of only 0.2 ng methadone/mg hair. How-

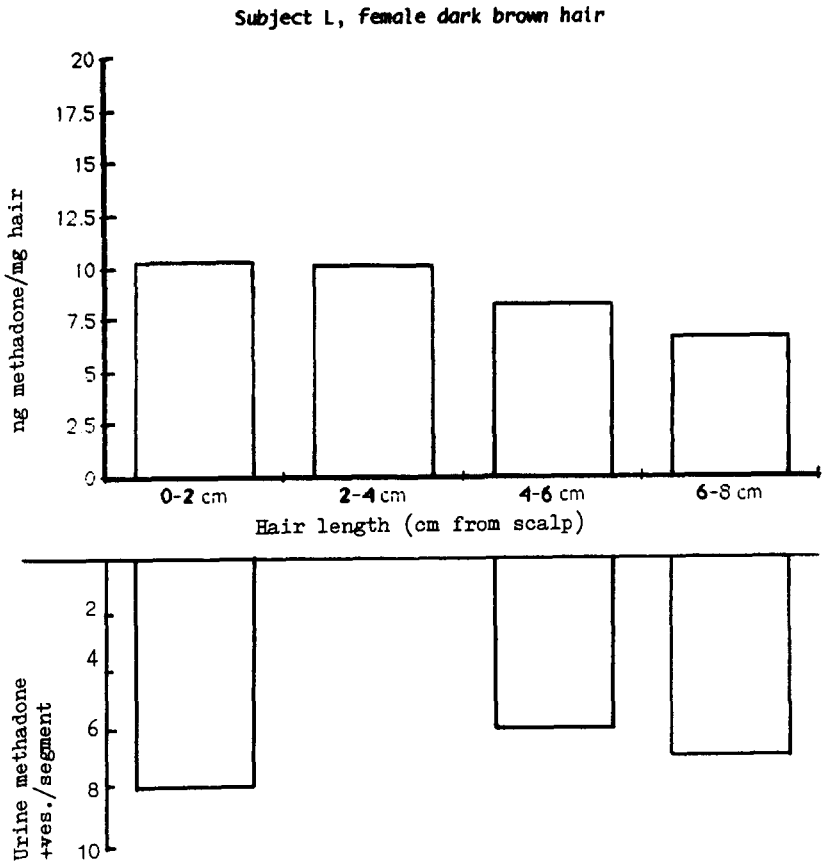


(a)



(b)

Fig. 3. (a–b).



(c)

Fig. 3. Patterns of methadone use for individual subjects (a) J, (b) K and (c) L by analysis of hair samples segmented according to different periods of time. Urine analyses, where available, represent total number of urine samples analysed per segmental period, their positive nature being indicative of methadone use.

ever, patients on a similar prescribed methadone intake gave concentrations in the range 1.9–6.4 ng methadone/mg hair. Subsequent cream peroxide treatment of a further sub-sample from subject H in the laboratory reduced the concentration to 0.11 ng methadone/mg hair. This is very close to the proposed level of cut-off deduced from the analysis of volunteers from the non-drug user population. On this evidence, it would seem that the continual treatment of hair by strong peroxide may preclude the detection of methadone by radioimmunoassay.

3.3. Methadone levels in segmented hair of known abusers

The concentration of methadone in the hair segments of known users, subjects J, K and L, were found to range between 1.42 and 10.63 ng methadone/mg hair. Random hair segments from known non-drug users assayed at the same time gave levels between 0.00 and 0.03 ng methadone/mg hair. This is below the determined negative cut-off. However, the positive nature of this range would probably not be due to methadone intake or cross reactivity, but more likely to non-specific binding (0.8%, 630 counts per min) and the sensitivity limits of the assay (0.5 ng ml^{-1} equivalent to 0.02 ng methadone/mg hair based on a hair sample of 30 mg). The concentration of methadone found in the hair segments at different distances from the scalp are shown together with results from cumulative urine assay, where available, as further indication of methadone usage (Fig. 3). Results from the segmental hair analysis of subjects J, K and L suggest a means of providing an historical account of drug usage over an extended period of time representing many months.

Subject J shows a decrease in the concentration of methadone in the hair over a 6 month period, the lowest value being in the hair segment representing most recent growth. This suggests a decreased methadone intake over the 6 month period. The subject self-reported a daily consumption of up to 110 mg methadone

on an irregular basis, with a reduced intake of 55 mg per day during the last month. Should cumulative urine results have been available throughout the period of the investigation, they may have given consistently positive results, without indicating the improvement in methadone dependence as based upon the self-report. The pattern from segmented hair analysis for subject K suggested an increase in the use of methadone over the last 2 months, from an apparant constant usage. Regrettably, there was no report available relating to illicit or prescribed methadone use for this subject. Sectional hair analysis for subject L covered a period of 8 months. During the period represented by the 6–8 cm hair segment, the subject was stable on a prescribed dose of methadone, 20 mg per day, although the occasional use of heroin was admitted. 6 months prior to hair sampling the subject underwent a reduction withdrawal programme from 25 mg methadone per day over a period of 10 days. However, shortly afterwards the subject was discharged and treatment on a prescribed daily dose of 25 mg methadone was resumed until the time of hair sampling. The segmented hair analysis regime appeared to corroborate the reported use of methadone and has demonstrated constant use since discharge for this individual.

In each of the above cases, the urine results represented the total number of urine specimens analysed for methadone during segmental time periods. The positive nature of all the cumulative urine analyses indicated that methadone was being used.

Compartmentalisation of hair samples provided a means of detecting drug abuse over many months, depending upon the length of the hair sample, and allowed an insight into the drug taking pattern of an individual in terms of it being constant, increasing, decreasing or abstinent.

3.4. Hair segmentation and detoxification

The concentration of methadone deposited in the hair of subject M undergoing a 10 day methadone detoxification programme was

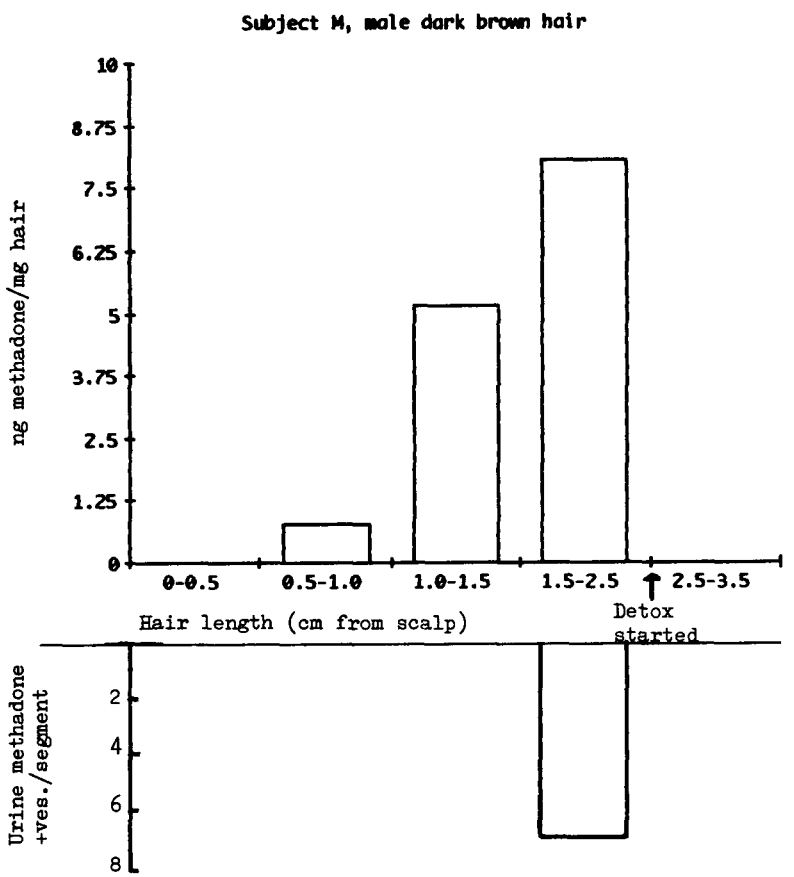


Fig. 4. Time profile of methadone concentration detected in hair segments together with methadone positive urine detection, following a methadone detoxification scheme, whereby a total of 426 mg was given in decreasing doses over a 10 day period.

studied. A total 426 mg dose of methadone, as linctus, was administered in decreasing daily dosages post-admission to the in-patient detoxification unit. A sample of hair was taken, as described earlier, 2 months after completion of the programme, and the most recent 3.5 months hair growth equivalent analysed. Urine specimens collected approximately every 3 days post-admission were qualitatively analysed for methadone by thin layer chromatography [28] for the following 78 day period. The concentration of methadone, as ng methadone/mg hair, detected in each hair segment together with the corresponding number of positive urines are shown in Fig. 4.

A urine specimen analysed qualitatively on admission was positive for morphine, barbiturate and cannabis. The presence of morphine was also detected in the hair segment representative of a month prior to admission, but the methadone content was below the pre-determined cut-off. There were no urine records available prior to the admission date.

The highest methadone concentration, 8.06 ng methadone/mg hair, was detected in the segment representing the monthly growth equivalent to post-admission, the total methadone dose having been given at the beginning of the month. The level of methadone in the hair decreased until methadone was no longer found to be present. However, based on the segmentation scheme adopted, the hair sample remained above the pre-determined cut-off for an approximate period of 48 days following the completion of the detoxification programme. Qualitative urine analysis remained positive for only 7 days post-completion. This fact demonstrates the complementary approach of the two modes of drug analysis, urine providing evidence of short term drug use whereas hair analysis indicates past, present, continuous or periodic drug use. Cone [12] demonstrated that it took morphine and codeine 7–8 days to appear in beard hair post-administration, at a time when drug levels in urine were no longer detectable. A dose response relationship between drug levels in beard and drug dose was also suggested. Beard and head hair grow at similar rates [4]. Results from the methadone detoxification, albeit limited, also suggest a dose response relationship between methadone levels in hair and dose of drug administered. Methadone dosing during detoxification was given over a period of 10 days, the dose on day 1 being 30 times stronger than on day 9. The more concentrated doses would be metabolised and appear at scalp level sooner than the weaker doses. This could explain the pattern of hair methadone concentration described in Fig. 4. Methadone was retained in the hair and segmentation was capable of providing historical records of drug intake.

3.5. Hair methadone concentration and methadone intake

The correlation between methadone intake (g per month) and the concentration of methadone detected in the hair (ng methadone/mg hair) is illustrated in Fig. 5. The study compared self-report and prescribed methadone intake, covering a period of 1 month prior to hospitalization, with the assay of the corresponding segment of hair growth of a group of 19 new admissions. Notwithstanding problems associated with self-reporting in terms of strict compliance, extra use, drug purity, accurate recall and routes of drug administration, it was considered ethically more feasible to study such a group of volunteers stabilised on prescribed

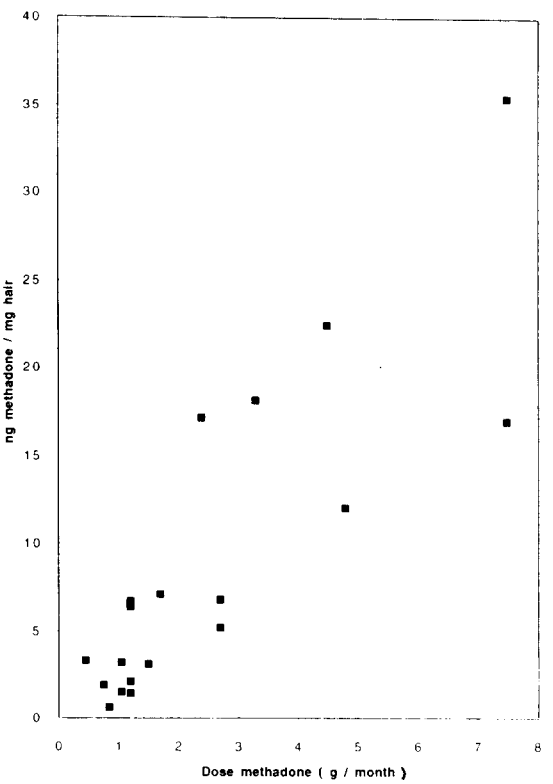


Fig. 5. Correlation between self-reported methadone use and found hair methadone concentration, based on patient self-report and prescribed methadone regimes.

methadone regimes than to administer an additive drug to drug-free volunteers. The present study of self-reported intake and the hair methadone content indicates that a correlation exists in terms of low, intermediate and high drug intake. Thus, it is possible to obtain information on the pattern of the drug use of an individual, in terms of it decreasing, increasing or remaining constant, through quantitative hair drug analysis. Hence, relative changes in drug use within an individual can be established by the analysis of hair segments. However, absolute estimates of methadone intake in relation to concentration detected in the hair would almost certainly be subject to individual biochemical variation.

3.6. Hair methadone extraction and analysis

Drug-free hair samples were spiked with known concentrations of methadone hydrochloride prior to extraction for quality control and recovery purposes in all assays involving the Coat-a-Count system. Hair samples from known methadone users and non-drug users were employed as quantitative controls. The recovery of methadone added as spike to washed control hair following acid extraction was 90, 79, 89, and 85%, respectively, for spiked concentrations of 75, 100, 400 and 500 ng ml^{-1} methadone.

4. Conclusions

The present studies indicate that the previously adapted Coat-a-Count radioimmunoassay system is suitable for the assay of methadone in human hair samples following acid extraction. The importance of pre-analytical washing has been demonstrated, and a cut-off level to distinguish between the methadone user and the methadone-free subjects has been established. It has been shown that drug levels of methadone in hair can be reduced with certain hair treatment procedures, although possibly not eliminated under reasonable conditions. Finally, a segmentation protocol has revealed the potential of hair analysis to provide an historical account of drug intake over many months, and provides evidence that a dose response relationship between hair drug levels and intake may exist within an individual, although absolute estimates may be subject to the rigours of biochemical individuality.

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